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Dispersal and Reproductive Competition in Mammals

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Dispersal and reproductive competition in mammals (by Liane Hobson)

Dispersal behaviour (DB) and reproductive competition (RC) are interrelated factors that are key in many evolutionary and ecological processes. DB dictates the density, dynamics and composition of populations. DB thus affects, for example, the risk of kin competition, level of RC and population longevity. RC impacts behaviours such as mating strategies and tolerance for conspecifics, which can affect DB. Despite the importance of DB and RC, their association and their causes and consequences are poorly understood. This thesis explores how mating system, competitor relatedness and maternal effects influence DB and RC in mammals.

Local mate and resource competition are ultimate causes of DB. Both are also key components of RC, and are associated with mating system. Particular mating systems are thought to cause a given dispersal pattern. Here, I explore hypothesised links between mating system and DB in mammals using a comparative approach. There was little evidence to support the proposed links between mating system and DB, excepting an association between unbiased dispersal and monogamy.

Intrasexual kin competition may influence both DB and investment in RC. In mammals, males usually compete for mates, and competition may occur before and/or after copulation. Males are expected to invest relatively less in RC when competing primarily among kin. Using phylogenetic analyses, I found evidence that ejaculate investment was greater when males are more likely to compete with kin, and that males may avoid kin when the level of pre-copulatory competition becomes high.

Population density is positively correlated with RC and dispersal propensity, and may cause maternal effects on many traits. I use bank voles (*Myodes glareolus*) as an experimental model to investigate maternal effects on traits related to DB and RC in mammals. I manipulated perception of population density under laboratory conditions by exposing adult females to social cues indicating 'high' or 'low' levels of same-sex conspecifics. In litters produced by these females, I found evidence of a maternal effect on sex ratio, but not litter size, pup growth rate or offspring weaning mass. Males born in litters produced females exposed to 'high' levels of social cues had higher rates of daily sperm production and larger epididymides, consistent with an adaptive maternal effect on reproductive traits. However, there was no evidence for a maternal effect on DB.

By providing new insights into associations between DB and RC in mammals, the work presented herein has potentially far-reaching impacts in evolutionary ecology, and provides a basis for future advances in understanding patterns of dispersal across diverse species.

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Sorry I couldn't finish writing before you had to go grandad, I hope I've done you proud.

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Chapter 1: General introduction

1.1. Chapter overview

In this chapter I will introduce the main topics studied in this thesis, with a particular focus on mammalian species. First, I will present an overview of the literature on reproductive competition in both males and females. Here, I will consider the costs and benefits of investment in reproductive competition, the evolutionary consequences of reproductive competition on reproductive traits, and I will describe how mating system is likely to be related to the level of reproductive competition. I will then introduce dispersal behaviour, which can be influenced by reproductive competition and is often associated with mating system (see Chapter 2). I will summarise the terminology used, techniques used to study dispersal, potential drivers of dispersal and the costs and benefits of the behaviour. I will then briefly introduce maternal effects, which are known to affect both reproductive traits and dispersal behaviour among other factors. Finally, I will briefly explain how animals can be used as models in studies before introducing the species used as a model in this thesis, the bank vole (*Myodes glareolus*). I will review the general ecology of *M. glareolus* before specifically focussing on reproductive competition and dispersal behaviour in this species. At the end of this chapter, I will provide an overview of the content of each of the subsequent chapters.

1.2. Reproductive competition within the sexes

Reproduction and survival are of critical importance in the lives of organisms. Indeed, biological adaptations can typically be explained in relation to competition for resources required to survive and reproduce, including mates and food [1,2]. Levels of competition are often greatest between members of the same sex (i.e. intra-sexually), as their resource requirements to maximise reproductive success are more likely to be similar [3–5]. In males, intra-sexual competition is typically related to ensuring access to sexually receptive females [3,4]. By contrast, intra-sexual competition between females is most often to secure access to resources required to breed besides mates, such as nest sites [3–5]. Competition between members of the same sex, or intra-sexual competition, constitutes a significant selection pressure in sexually reproducing taxa [6]. Here, I summarise some of the literature on the costs, benefits and evolutionary consequences of reproductive competition, and factors influencing the level of reproductive competition in each sex, although I focus particularly on males.

1.2.1. Sexual selection

Sexual selection is a form of natural selection which includes aspects of competition both between and within the sexes (i.e. inter- and intra-sexual competition, respectively). Sexual selection was first characterised by Darwin [2], who asserted that it was reliant on traits that confer a reproductive advantage to some conspecific individuals of the same sex. Darwin [2] focussed primarily on the impact of sexual selection on males, and identified both female mate choice and intra-sexual competition between males as the most important causes of sexual selection [7]. Many subsequent studies on reproductive competition and sexual selection also focussed on the adaptations and evolutionary consequences in males (discussed in e.g. [8]). More recently, studies have also begun to consider the effects of intra-sexual competition in females [6,8]. As such, reproductive competition in females is relatively understudied.

1.2.2. Costs and benefits of reproductive competition

1.2.2.1. Costs

Participation in intra-sexual competition for resources, including mates, is costly with respect to energy, time and risk. Individuals often invest significant levels of energy in the defence of desired resources, participation in overt or subtle contests and the development of traits that may confer a benefit when competing. The latter may include increased investment in traits including gametes (see Section 1.2.4.2. for information on sperm competition) and body size, as well as investment in the development of armaments (reviewed in [9] for males and [8] for females; Sections 1.2.3. and 1.2.4.1.).

Males may spend a significant proportion of their time budget during the breeding period locating and/or defending primary access to a receptive female, or females [10,11]. During the periods when females are receptive, males typically invest less time than normal in other activities, such as foraging [10]. This can have a marked negative effect on the future reproductive success of males [10,11]. For example, Preston *et al*. [11] found that male Soay sheep (*Ovis aries*) spent 18% of their time checking the oestrus status of females during the rut. Males did not forage at ordinary levels during the rut, resulting in significant weight loss. Low quality males typically weigh less than other males, and the weight loss during the rut is more likely to negatively influence their future reproductive success [11].

Females may also dedicate a significant portion of time defending desired resources. Females are likely to compete for resources related to reproduction other than mates [12]. In some

social systems, females may invest more time than males defending such resources. For example, in the spotted hyena (*Crocuta crocuta*) females spend more time than males on activities related to territorial defence, such as patrolling territory borders and scent marking [13,14].

Finally, overt contests can pose a significant risk to individuals. Although females may participate in direct, aggressive intra-sexual contests, this behaviour is more common in males [8]. Males can incur significant injuries during male-male contests, which can influence future reproductive success and survival [15,16]. Males also risk immediate mortality, as fights can be fatal [17,18]. Males may also be impacted by injuries to conspecifics. For example, in lions (*Panthera leo*), reproductive success is dependent on the ability of a group of males (who are often related) to secure access to females [19]. In such species, the loss of one male can significantly impact the reproductive success of the remaining group members, so the consequences of overt contests can impact multiple individuals. Male-male contests may also pose a significant risk to females and offspring, as both may be killed or injured when males compete [20,21]. Females that participate in overt contests may be expected to face similar risks to those experienced by males.

1.2.2.2. Benefits

While intra-sexual competition can be extremely costly, individuals that compete successfully gain significant benefits. Males can maximise their reproductive success by outcompeting other individuals and thus securing access to females. Males that do not win contests may still be able to reproduce, but will have a lower level of reproductive success [22]. Similarly, females may maximise reproductive fitness by gaining access to high quality resources [5,6,8]. Females that do not gain access to the best resources may still gain some reproductive success by settling in low quality habitat [23]. Males or females that are completely excluded from resources including mates may emigrate to gain reproductive opportunities in another area, which is costly ([24]; Section 1.4.5.1.). Given the high overall benefit, it is unsurprising that individuals invest significantly in competing intra-sexually to secure resources [8,22].

Intra-sexual contests can also be beneficial to members of the opposite sex, and possibly the population as a whole. Individuals that acquire access to resources, including mates, and thus reproduce successfully, are the most competitively able in the population. Competitive ability is typically a reliable indicator of the quality of an individual [25–27]. Intra-sexual

competitions thus allow individuals to identify and mate with the best quality individuals, which will maximise the fitness of offspring [28,29].

1.2.3. Evolutionary consequences of reproductive competition in females

Although reproductive competition between females typically occurs to secure resources other than mates [3–5], reproductive competition for access to mates has been observed [6,8,30]. There is also evidence that competition for resources and mates overlap. For example, in some cases females are able to obtain access to a limited resource by competing and securing sole access to mates that control resources. Thus, at least some intra-sexual competition for mates may ultimately be driven by competition for resources (reviewed in [8]). Reproductive competition in females has been found to cause many adaptations including aggressive behaviour, weaponry, sexual signalling and other, more subtle social behaviours [8]. Here, I explain just some of the evolutionary consequences of reproductive competition in females.

Female mammals may participate in overt, direct and aggressive intra-sexual contests, although such behaviour is more common among males [8]. In males, the levels of aggressiveness, physical strength and investment in armaments are key determinants of competitive ability, and there is evidence that the same may be true for females [8].

The reproductive success of females can be limited by the inability to access resources in sufficient levels to breed. Individual or groups of females may participate in territorial defence, which is often associated with high levels of aggressive behaviour, to secure access to limiting resources [8,31,32]. Female territoriality has been observed in several species, including microtine rodents. The reproductive success of female microtine rodents is significantly, negatively affected when access to food resources is limited during the breeding season. Accordingly, territoriality of female microtine rodents becomes particularly evident when food resources are sparsely or discontinuously distributed and/or slow to regenerate [32,33].

The level of access to resources can be somewhat determined by resource defence ability in both solitary and group-living species. In group living species with social hierarchies, accessibility of resources may also be influenced by the dominance status of an individual. Dominant individuals typically have greater access to high quality resources, and thus greater reproductive success, than subordinates (summarised for cooperatively breeding mammals and birds in [34]). For instance, in several species of baboon, dominant females are subject to lower levels of interruption during feeding bouts, are more likely to be able to access high

quality resources nearest to their location and can more freely explore resources when feeding [35–37]. Dominance can also significantly impact the reproductive fitness of young, with the young of more dominant individuals more likely to survive and/or to reproduce more successfully [38].

Dominance in females, as in males, is often related to age, aggression and/or body size, with older, more aggressive and larger (and thus stronger) individuals typically being more dominant. For example, in feral ponies (*Equus caballus*) [39] and African elephants (*Loxodonta africana*) [40] the eldest and largest females are dominant to other females. Older females are also more dominant in chimpanzees (*Pan troglodytes*) [41] and bottlenose dolphins (*Tursiops truncatus*) [42], but aggression between females is low in these species (reviewed in [8]). Where dominance is related to body size, larger females have an adaptive advantage, so intra-sexual competition in females may lead to selection for increased body size as well as greater aggression.

Armaments, including antlers in red deer (*Cervus elaphus*) [43], are thought to have evolved primarily for use by males in intra-sexual contests to secure mates (see Section 1.2.4.1. for further details). Females in some species also possess weaponry, although it is seen relatively infrequently (see [8] and references therein). As in males, females may use antlers or horns in predator defence and/or in inter-female contests, although direct evidence is rare in mammals [8,44–46]. Female caribou (*Rangifer tarandus*) with larger antlers were more likely to gain access to limited food resources [47]. Moreover, horns were found to be of major importance in determining dominance relationships in new social groups in domestic cattle (*Bos Taurus*) [48]. Thus, at least in some mammals, reproductive competition may lead to greater investment in armaments to improve competitive ability. There is comparatively more evidence for the use of weaponry in female-female contests in other taxa, including insects [30]. Indeed, there is recent evidence from the dung beetle *Onthophagus sagittarius* that females with greater horns are more competitively successful and that there is significant selection on this trait [30].

One of the better studied consequences of intra-sexual competition among females is increased rates of female dispersal. Banded mongooses (*Mungos mungo*) and meerkats (*Suricata suricatta*) are cooperatively breeding species in which relatively few individuals dominate reproduction [49,50]. In each species, groups of females and sometimes mixed sex groups, can be forcibly evicted by breeding females due to high levels of reproductive competition [49,50]. If the probability of female is dispersal is greater due to increased levels

of intra-sexual competition, then any associated adaptations could be considered indirect evolutionary consequences of reproductive competition. I will discuss possible drivers of female dispersal, including competition for resources, in Section 1.4.5. and Chapters 2 and 5.

Other traits influenced by female intra-sexual competition, including prenatal investment in young (see [51] and references therein), signalling and more subtle behaviours, remain relatively understudied compared to those discussed above [8].

1.2.4. Reproductive competition in males

Reproductive competition has caused the evolution of several competitive strategies in males. The evolution of these strategies is relatively well studied. The strategies used by males can be broadly divided into two categories: competition before mating and competition after mating (pre- and post-copulatory competition, respectively). Pre-copulatory competition is typically focussed on securing access to receptive mates. To access mates, males usually engage in the overt, costly contests (as described in Section 1.2.3.). Post-copulatory competition only occurs if females mate with multiple males. When females mate multiply, the sperm of several males will compete to fertilise an egg, or ovum. This is termed sperm competition (as defined by Parker [52]). In this section, I will outline the strategies used by males in pre- and post-copulatory competition, and the evolutionary consequences of each.

1.2.4.1. Evolutionary consequences of pre-copulatory competition

The evolutionary consequences of pre-copulatory competitions in males mirror those described for females above. I will describe the consequences for males briefly here. More details can be found in Chapter 3.

Pre-copulatory competition between males, as in females, can lead to highly aggressive, overt contests [8,12,43,53]. These contests can be used to establish dominance hierarchies in males [43,53]. Dominant males typically have the greatest levels of reproductive success and are often both the most aggressive and largest males [11,54–57]. Dominant males may control access to females throughout the breeding period in order to maximise reproductive success [58]. However, the fertility of females typically varies throughout the breeding season, and males that breed with females at their most fertile are more likely to sire offspring [55,59]. Dominant males may therefore defend females only at the peak of their fertility [55,59]. Where this occurs in group-living species, all resident males may mate with females, but dominant males will still have greatest reproductive success

The order of male mating can also be important for reproductive success in some species (discussed in [60]). In some species males queue to access females, and the order of males in the queue may be determined according to dominance, although males may queue-jump to improve reproductive success [61]. This is most often observed in primate species, such as savannah baboons (*Papio cynocephalus*) [61]. While dominance is determined in pre-copulatory competition, the advantage of dominant males where competitors queue is actually related to the influence of mating order on success during post-copulatory competition (Section 1.2.4.2.). As such, dominance may confer an advantage in both pre- and post-copulatory competition. The significant benefits associated with dominance can cause intense male-male contests for dominance, potentially resulting in selection for large size and high levels of aggression.

As mentioned in Section 1.2.3., armaments in mammalian species are thought to have evolved principally for use in male-male competitions to secure access to females [8]. Armaments, including horns and antlers, have been shown to confer a significant advantage during combat, and are often associated with increases in overall body size. Males with the largest weaponry and greatest body size typically outcompete other contenders and gain greatest reproductive success [11,43]. Body size can also be positively correlated with competitive ability, and thus reproductive success, in the absence of weaponry (discussed in [62] for species in several taxa). The benefit of relatively increased male size can promote the evolution of sexual size dimorphism (SSD) in which males are significantly larger than females (i.e. male-biased SSD) [62]. Overt inter-male contests to secure mates, or at least the resources in order to obtain mates, is common in mammals and may have led to the widespread exhibition of male-biased SSD [62,63].

Overt inter-male contests can act as a reliable indicator of male quality as the best quality males are expected to be more competitively able, and thus to be more likely to win. Males may also use other signals to convey their relative quality. Such signals are likely to be reliable, as they are often limited by some feature related to male quality [25,64]. These signals are typically less costly for males than participation in overt contests, particularly as there is less risk to males. Nevertheless, males may invest a significant amount of time and energy on signalling, which may include visual, scent or auditory cues (discussed in [64]). The capacity for males to participate in such activities is limited by energy availability and ability, both of which are likely to be higher in better quality males [64]. For example, in red deer (*Cervus elaphus*) males can use antlers in contests to secure access to a group of females [43]. However, males may also use a behaviour termed ‘roaring’ to reliably indicate their quality

and to reduce the need for competitive fights. Larger, better quality males are able to produce longer, louder roars because they have bigger chest cavities and greater energy reserves [64]. This means that better quality males should be victorious in roaring contests. Roaring contests between males thus offer a means by which the relative quality of each male can be assessed without the need for competitive fights as males may only be expected to fight where there is no clear victor in roaring contests [64]. This minimises the risk of potential injury to males whilst competing for mates.

In other species, signals of quality are primarily visual. Visual cues may consist of repeated movement of appendages or differences in colouration. For example, in mandrills (*Mandrillus sphinx*), males exhibit two primary phenotypes; 'fatted' and 'non-fatted'. Male secondary sexual characteristics include bright red and blue colouration on their face, genitalia and rump. Fatted males have brighter colouration, occupy relatively high social ranks and have greater reproductive success than non-fatted males [54].

1.2.4.2. Evolutionary consequences of post-copulatory competition

Sperm competition was initially defined by Parker [52] as the competition between the sperm of multiple males to fertilise an ova [52]. In this section, I will provide an introduction to some of the evolutionary consequences of sperm competition in mammals. Evolutionary consequences of sperm competition have been identified primarily for three phenotypic aspects of mammalian males: genital structure, sexual behaviour and reproductive physiology [65,66]. The literature on this subject is extensive, but I focus here primarily on those factors most relevant to the work in this thesis. I will then present an overview of some relevant sperm competition theory and predictions for mammalian males.

1.2.4.2.1. Timing of sperm production and mating

The advent of DNA tools to assess paternity in litters has shown that multiple mating in females, and thus sperm competition, is more widespread in mammals than previously believed [67–70]. Multiple paternity of litters has now been detected in polytocous mammalian species [60,66,71–73]. In most species, females are only sexually receptive for a short period, known as oestrus [74]. Sperm are generally short lived [75], and males typically match sperm production to the receptivity of females [74]. Bats are a notable mammalian exception to the production of short-lived sperm, as females can store sperm in the reproductive tract over winter and use it to fertilise ova in the spring [76,77]. Generally though, as suggested above, the timing and relative order of mating can have a significant impact on relative reproductive success [55,59,61].

1.2.4.2.2. Sperm production, ejaculate investment and the size of reproductive organs

The size/mass of testes relative to body mass is generally considered to be a reliable indicator of investment in sperm competition in a variety of taxa (mammals: cetaceans [78], rodents [79,80], bats [77,81,82], primates [83], carnivores [84]. Other taxa: birds [85], invertebrates [86], fishes [87], amphibians [88]). Parker [89] compared sperm competition to a raffle, with those contributing a greater numbers of 'tickets' (i.e. sperm) more likely to win. Where the probability (or risk) of sperm competition is high, males are therefore expected to invest greater numbers of sperm in their ejaculates [89]. The sperm production capacity of males is determined by testes size, with males with larger testes able to produce greater numbers of sperm and to include high levels of sperm in ejaculates [83].

The hypothesis outlined above (i.e. that larger testes confer an advantage in sperm competition because they enable greater levels of sperm in ejaculates) is termed the 'numerical sperm competition hypothesis'. This hypothesis has been widely accepted compared to possible alternatives. However, some authors [90] claim that an alternative hypothesis, namely 'the male mating rate hypothesis', should not be dismissed. This latter hypothesis proposes that males with proportionately large testes have an advantage as they can produce more sperm and thus ejaculate a greater number of times. Under the male mating rate hypothesis, males are expected to produce smaller ejaculates than those predicted by the numerical sperm competition hypothesis. However, males will be able to mate relatively more frequently with multiple different females, which could confer an advantage in sperm competition. The relative evidence for each hypothesis is debated in great depth in Vahed and Parker [90], who argue that the evidence often cited in support of the numerical sperm competition hypothesis could equally support the male mating rate hypothesis. Overall though, there is little reason to suggest that the male mating rate hypothesis should be favoured over the numerical sperm competition hypothesis, and most authors argue in favour of the latter, more widely accepted hypothesis. Accordingly, I favour the latter hypothesis throughout this thesis.

There are several aspects of the ejaculate, besides the number of sperm, that can influence the likely success of males competing post-copulation. The morphology of spermatozoa is thought to be related to likely fertilisation success and sperm competition is thought to be a driving force in the diversification of sperm morphology (reviewed in [91]). For example, sperm that reach an ovum first are more likely to fertilise it, so morphological adaptations to

increase the speed of sperm are likely to cause an advantage during sperm competition [74,91].

Males may also maximise their share of paternity by reducing the likelihood of sperm competition. Males may minimise the risk of sperm competition by preventing the sperm of other males from entering the reproductive tract of a female he has mated with. This can be achieved using pre-copulatory behaviours, including overt contests, which can cause other males to become at least primarily excluded from females [12,92]. Males may also deter other males from mating with the same female using mate-guarding [93,94]. Mate-guarding is associated with selection for increased body size in males and male-biased SSD, as with traits related to pre-copulatory competition [95,96] (further details in Section 1.2.4.1. and Chapter 3). Alternatively, or additionally, males may use copulatory plugs, which may prevent the sperm of males that subsequently mate with the female from fertilising ova, although this is only one hypothesis for their function [95,97–99]. Copulatory plugs have been observed in many mammalian species including rats, guinea pigs and various non-human primates [100]. Copulatory plugs are formed when seminal fluid coagulates in the female reproductive tract, causing it to become temporarily blocked [100,101]. Clotting of seminal fluid occurs due to the coagulation of proteins produced in the seminal vesicles of males. Coagulation is induced by enzymes, specifically transglutaminases, that are secreted primarily by the anterior prostate gland [100,101]. Males may increase investment in the components required to form a copulatory plug when females are likely to mate multiply. Increased investment in copulatory plugs can cause the mass and/or size of seminal vesicles to be relatively large in relation to body size [102] (more details Chapter 4).

Relatively increased investment in pre-copulatory competition is associated with greater male body mass or male-biased SSD, but this is not the case when males increase investment in post-copulatory competition [95]. I discuss this further in Chapter 3.

1.2.4.2.3. Sperm competition games

In early studies the costs of sperm production were thought to be negligible, enabling males to produce as many sperm as desired with no trade-off on other traits [103]. This early view is reflected in a quote from Dawkins [104], who stated that 'the word excess has no meaning for a male'. More recently, it has become clear that the production of sperm is costly to males, and trade-offs with other traits are common [91,105–109]. There is considerable evidence that males can vary the amount of sperm in a given ejaculate, otherwise known as their sperm allocation [103,105–115]. Several empirical and theoretical studies have

considered which factors could cause males to alter sperm allocation (see [103,105–117] and references therein). Examples of factors that affect sperm allocation include the relative risk and intensity of sperm competition [87,89,105,107,112,118], the roles of males [73,106,114,119,120], the mating status of females [107], the level of pre-copulatory competition [109] and the relatedness of competing males (discussed in [117], and in Chapter 3). Sperm competition games are evolutionary stable strategy (ESS) models developed to investigate optimal investment in ejaculates under different scenarios [107,109,113–115,117,120].

Sperm competition games each assume that males have a limited amount of energy which can be invested in reproductive competition [89,109]. Males are expected to allocate this energy to pre- and post-copulatory competition strategies in a manner that should maximise their reproductive success [89]. Models of sperm competition games therefore adopt an evolutionarily stable strategy (ESS) approach, in accordance with evolutionary game theory [89,109,121–123]. There is relatively little direct evidence of the trade-off between investment in pre- and post-copulatory competition in mammals , but there is some evidence from other species groups including amphibians and beetles [109,124–128].

Here, I broadly describe some of the best studied models, and the evidence for each in mammals. I limit the discussion to sperm competition games based on the risk and intensity of sperm competition and the raffle type and roles of males. Later in this thesis, I also test the predictions of a set of models on sperm competition games outlined by Parker [117]. Further details of these models and the evidence in mammals are provided in Chapter 3.

Models that predict sperm allocation according to different levels of 'risk' and 'intensity' of sperm competition are perhaps the best studied. Indeed, there is extensive theoretical [89,107] and empirical evidence [103,105] that the risk and intensity of sperm competition affects the level of post-copulatory investment by males in a wide variety of taxa. The risk of sperm competition can be defined as the probability that the ejaculate of a given male will compete with that of another [105,107,110]. As mentioned previously, males that are more likely to experience sperm competition (i.e. have a greater risk of sperm competition) may outcompete rivals by investing more in sperm production, which can have consequences for testes mass [90,107]. Males are expected to allocate the least amount of sperm when there is no risk and investment is expected to increase with the relative risk [107]. Empirical evidence to support the predictions from models based on the risk of sperm competition is relatively consistent. Sperm allocation has been found to increase with the probability of

sperm competition in several species groups including fruit flies [129], bush crickets [130], butterflies [131] and mammals [132,133].

The intensity of sperm competition relates to the number of rivals a given male will compete with [89,105,110,118]. In models of the intensity of sperm competition, males always compete with at least one rival to fertilise the ova of a female [107,118]. As such, models considering the effects of sperm competition intensity always assume that the risk of sperm competition is high [89,103,107]. At a species level, testes size, and thus overall ejaculate investment, is predicted to increase with increasing sperm competition [134]. However, this is not the case at the individual level. In ESS models considering the effect of sperm competition intensity on sperm allocation, it is predicted that individual males will invest high levels of sperm when intensity is low (i.e. there is only one rival) and that investment will decrease with increasing intensity [103,105,107,110,112,118]. Investment is predicted to decrease with increasing intensity as males are less likely to gain a high share of paternity when intensity is high, so increased investment will not necessarily significantly improve reproductive success [103,105,107,110,112,118]. There is conflicting evidence from empirical studies investigating the effect of sperm competition intensity on sperm allocation [103,105,110]. Three relationships between ejaculate investment and sperm competition intensity have been detected [110]. Ejaculate investment may decrease with increasing intensity, consistent with the predictions of intensity models, as found in the cricket *Gryllus veletis* [105]. Ejaculate investment has also been found to increase with intensity, as in the small white butterfly *Pieris rapae* [131]. Lastly, ejaculate investment may be independent of sperm competition intensity, as suggested for the rainbow darter, *Etheostoma caeruleum* [135]. There is very little evidence from mammals. The first empirical study in mammals was completed by DelBarco-Trillo and Ferkin [110] in the meadow vole, *Microtus pennsylvannicus*. Ejaculate investment in *M. pennsylvannicus* was found to vary in accordance with the predictions of ESS models, with investment decreasing with increasing intensity. Further evidence on other mammalian species is necessary to determine whether the effects of sperm competition intensity are likely to be consistent.

As mentioned above, sperm competition can be likened to a raffle [89]. In general, males that put more 'tickets' (i.e. sperm) into the raffle are expected to be more successful. However, this is only true if the raffle is 'fair'. The raffle is fair if the sperm contributed by each male are equally likely to fertilise ova [120]. The raffle is unfair if the sperm from one male has an advantage, or is favoured, over the sperm of another [120]. Models that assume that all males are equally likely to be successful are said to describe 'fair raffles', otherwise models

are said to describe 'loaded raffles' [120,136]. The role of a given male (i.e. whether it is favoured or not) might be assigned randomly or non-randomly. The role would be random if, for example, males mated with females in a random order and mating order influenced likely success. Alternatively, mating order will be non-random if a given male or males with particular phenotypes always have greater levels of success (for further details see [106]). For example, roles in savannah baboons (*P. cynocephalus*) are non-random as dominant males tend to mate last, and thus gain a greater share of paternity [61]. Males may, or may not, have information about their role [120,136].

The ESS strategy of ejaculate investment in models considering different types of raffles and roles depends on the information available to males and whether their roles are random. For example, if males have information on their role but roles were assigned randomly, then sperm allocation is expected to be equal between the two rivals regardless of the raffle type. Males are also expected to invest equally in ejaculates if roles are non-random and the raffle is fair. However, if the raffle is loaded and roles are non-random then the male that is favoured should contribute less sperm, whilst still gaining the majority of paternity (see [120] for more details).

There is relatively little empirical evidence from mammals to support the predictions of raffle and role models of sperm competition. Lemaître *et al.* [119] completed tests of these models using the bank vole, *Myodes glareolus*. Dominant males were found to invest more in their ejaculates than subordinate males. This suggests that, contrary to the predictions of raffles and roles models, favoured (i.e. dominant) males invested more in ensuring reproductive success, and that subordinate males do not compensate for their less favoured role.

1.3. Factors influencing levels of reproductive competition and investment strategies in both sexes

Several factors may influence the level of reproductive competition, and thus relative investment in particular strategies. Here, I discuss some of these factors and how they can influence the investment strategies of individuals. I focus particularly on the effects of population density and the mating system of species, as these will be of primary importance throughout this thesis.

1.3.1. Population density

Population density is measured as the number of individuals in a population per unit area (which can be landmass or water, dependent on the species) [137]. If population density is

high, then more animals will compete for the same resources, including those required to reproduce successfully [138–140]. Investment in reproductive competition is therefore likely to be greater when population density is high. Population density can affect members of both sexes, although not necessarily in the same way. In mammals, males are more likely to compete for mates and females are more likely to compete for resources other than mates [8,24]. Increased population density may therefore cause males to invest more in mate competition and females to invest more in resource competition. Higher levels of investment may cause traits related to reproductive competition to become more exaggerated. For example, at higher population densities, females may be more likely to mate multiply, causing the risk of sperm competition to be increased [134]. The increased risk of sperm competition may then result in higher levels of investment in sperm production, and thus greater testes size relative to body mass [82,86].

The effects of population density on reproductive competition may also influence other factors, such as general population dynamics, which can also impact phenotypic traits. When population density is high, some individuals may be excluded from a required resource [24]. This may drive the dispersal of at least some members of the population [24,141–143]. The impact of reproductive competition as a driver of dispersal behaviour will be discussed in detail in Section 1.4.5.

1.3.2. Mating system

Mating system and reproductive competition are highly interlinked. Indeed, mating system can be said to arise from competition in, and consequent strategies adopted by, each sex. Conversely, the level of competition experienced by each sex is influenced by mating system. Here I will describe how mating system is classified before introducing how mammalian mating systems develop and how mating system could influence the level of reproductive competition experienced by each sex.

1.3.2.1. Classification of mating system

In this thesis, as in most previous literature, mating system is defined according to the relative number of mates each sex can accumulate [144]. Mammals can adopt one of four mating systems. If males mate multiply whilst females mate with only one male, the species is considered to be polygynous. The majority of mammal species identified to date are polygynous [24,142,145]. If females mate multiply but males do not then the species is classified as polyandrous this is possibly the rarest mating system among mammals ([24,142,145]; Chapter 2). Species are considered to be promiscuous if both males and

females mate multiply, whilst in monogamous species members of each sex mate at least primarily with one partner.

1.3.2.2. How mammalian mating systems arise

Emlen and Oring [144] developed a theory for the evolution of mating systems across animal species. Broadly, they suggested that mating system is influenced by the defensibility of mates and other resources required for successful reproduction. Models suggested that the defensibility of mates and resources is related to their spatial and temporal distribution. If resources, including receptive mates, are distributed equally in space and time, then individuals are unlikely to be able to monopolise them. If resources are instead clustered, then defensibility becomes possible. For example, individuals were expected to be able to monopolise more and/or better quality resources when they are unequally distributed. Similarly, as the operational sex ratio (OSR, the ratio of reproductively active males and females) is influenced by spatial and temporal availability of the limiting sex, mates should be more defensible when OSR is skewed. Models by Emlen and Oring [144] indicated that for monogamy to evolve, then individuals had to be able to economically defend access to a mate. By contrast, there were two 'pre-requisites' for the evolution of multiple mating (i.e. polygamy). The first was the economic defensibility of multiple mates, which could depend on the length and synchrony of sexual receptivity in the limited sex. For example, if sexual receptivity of females is highly synchronous, then a male is unlikely to be able to monopolise several mates. The second pre-requisite was that individuals could exploit the environmental potential for polygamy. The ability of males to use this potential is largely dependent on paternal care, with those that provide care less likely to be able to adopt polygamy.

Clutton-Brock [12] specifically considered the evolution of mating systems in mammalian species. Consistent with the previous findings in Emlen and Oring [144], he deduced that male mating behaviour was related to paternal care and the defensibility of females. Mate defensibility was dependent on the size and stability of female groups and female ranging behaviour.

In mammals, males typically defend mates whilst females typically defend resources related to reproduction, although there can be variation between species [12]. Moreover, females typically provide care whilst paternal care is rare [12,146,147]. Clutton-Brock [12] predicted that mammalian species would exhibit obligate monogamy where paternal care was necessary for successful reproduction or to significantly improve breeding rate. Where paternal care was not necessary, mating system was instead expected to be dependent on

female behaviour. Specifically, Clutton-Brock [12] expected that if the ranges of individual females were sufficiently small to be defensible by males, mating system would be either facultative monogamy or polygyny, dependent on whether males were able to defend one or multiple ranges, respectively. If males could not defend female ranges, for example where female ranges were large, then males may instead defend female groups, but only where group stability is high. If female groups could be defended, the number of males that breed with those females was thought to depend on group size, with larger groups more likely to contain multiple breeding males. In some species, males may not be able to economically defend either female ranges or females. In such species, males are predicted to defend territories and mate with females that enter those territories. The spatial distribution of such territories should depend on the dispersion of females, with territories more likely to be clustered where female density is high. If, by contrast, the dispersion of females is unpredictable, males may rove to locate females and defend them whilst in oestrus [12].

Given that the factors influencing mating system can vary both spatially and temporally, mating system can vary not only between species, but also between populations of a species in different areas and within populations over time.

1.3.2.3. How mating system influences reproductive competition

The mating system of a species can influence the nature, timing and level of reproductive competition for individuals in both sexes. As such, the reproductive strategies of individuals can vary according to mating system. This is particularly evident in males. Males divide their total reproductive investment between attaining mates and successfully fertilising ova [109]. In some mating systems, males may maximise their reproductive success by investing primarily in pre-copulatory competition. However, in other mating systems, investment in post-copulatory competition is relatively more important.

In monogamous species, individuals form male-female mating pairs and the OSR is approximately 1:1. An equal OSR is typically associated with low levels of competition within and between the sexes. This means that sexual selection is minimal in monogamous species [144]. As such, monogamous species usually exhibit size monomorphism (i.e. no SSD) and low intra-sexual aggression. Accordingly, investment in pre-copulatory competition is typically lower in monogamous species than in species with other mating systems [148]. Some species are strictly monogamous. For example, Kirk's dik-dik (*Madoqua kirkii*) exhibits a high level of fidelity [149]. However, in most 'monogamous' species both males and females seek extra-pair copulations [150]. Whilst females of monogamous species are perhaps less

likely to mate multiply than those in species with other mating systems, males may experience low levels of sperm competition where females seek extra-pair copulations. As the levels of sperm competition are likely to be low in monogamous species, males do not typically exhibit traits associated with post-copulatory investment, such as proportionately large testes [108,151–153].

Males of polygynous species can maximise their reproductive fitness by securing and maintaining access to several females. The resultant high incidence of pre-copulatory competition in polygynous species often leads to the development of armaments. For example, in red deer (*Cervus elaphus*) males develop antlers that they use to outcompete other males and attract females [43]. Males are less likely to be able to effectively monitor large groups of females, so extra-group paternity of offspring is more likely in larger groups [92]. Although females can seek extra-pair copulations, group males are likely to retain the majority of paternity without significant investment in post-copulatory competition [92]. Thus, males are likely to gain greater reproductive success by investing in pre-copulatory competition than post-copulatory competition. Males pf polygynous species therefore tend to invest more in pre-copulatory than post-copulatory competition [128,152,153].

Both males and females mate with multiple individuals in promiscuous species, so males experience relatively high levels of sperm competition and invest highly in their ejaculates as a result [109,117]. As sperm competition is more likely in promiscuous species than in species with other mating systems, investment in post-copulatory competition is relatively higher in polygynous species. Males of promiscuous species may invest in pre-copulatory competition if the number of accessible females is low, for example due to high female dispersion (discussed in e.g. [95]). However, because males are more likely to maximise reproductive success by investing in post-copulatory competition, investment in pre-copulatory competition is likely to be lower than in males of polygynous species [11,109,128].

1.4. Dispersal behaviour

Dispersal behaviour is broadly defined as a permanent movement between groups and/or areas ([142,154]; see Section 1.4.2. for more details on the definitions of dispersal behaviour). Reproductive competition and dispersal behaviour are highly interrelated (explored in detail in Chapters 2, 3 and 5). Dispersal behaviour can influence aspects of reproductive competition. For example, reproductive competition may only occur between relatives if kin do not disperse separately. Similarly, high levels of reproductive competition can drive the dispersal of some individuals (Section 1.4.5.). Because of these close

interrelationships, relationships between dispersal behaviour and reproductive competition are thought to be particularly important in understanding dispersal [141], and are central to the research completed in this thesis. One of the factors that is most commonly associated with dispersal behaviour is the mating system. The relationships between the mating systems and dispersal behaviour have been discussed for over 40 years [24], and yet they remain poorly understood (explored in Chapter 2).

I begin this section by discussing the importance of studying dispersal behaviour and clarifying the terminology used both in the literature and in this thesis. I then detail different methods of studying and quantifying dispersal, potential drivers of dispersal and the potential costs and benefits of the behaviour.

1.4.1. The importance of studying dispersal behaviour

The dispersal behaviour of a species determines which individuals remain in the population and which leave. As a consequence, dispersal behaviour dictates the composition of a population [155]. Dispersal is widely recognised as a key factor influencing several evolutionary and ecological processes across a wide variety of taxa [142,145,154,156–159]. For example, dispersal behaviour affects the capacity for range expansions [145,157], gene flow [160], population dynamics [161] and the ability of populations to respond to environmental change [157,162]. Understanding the causes and consequences of dispersal behaviour is unquestionably vital in species conservation and species control, both of which have important economic implications. In this thesis, I focus on mammals, but the importance of dispersal is ubiquitous across all animal taxa.

1.4.2. Definitions of dispersal behaviour

The absence of dispersal is termed ‘philopatry’. Animals that do not disperse at any point are regarded to be ‘philopatric’. Individuals are considered to be ‘resident’ in a population if they remain philopatric or have settled in a population after dispersal [24,141–143]. These terms are consistent throughout the literature. Historically though, and in stark contrast to these well-established terms, there has been no clear definition of what constitutes ‘dispersal’ [145]. The definition used has varied according to the species under study, the author(s) of the work and the particular trait considered [24]. The lack of consensus regarding a definition has severely hindered the progression of an understanding of dispersal behaviour [142,145]. Here I describe how different forms of dispersal behaviour are defined and how the terminology used has changed over time.

1.4.2.1. Distinctions due to the social behaviour of species

Relatively early studies of dispersal behaviour focussed on solitary living species (i.e. species in which individuals forage and travel independently [163,164]). Dispersal in solitary living species often constitutes emigration away from both familiar conspecifics and well-known areas simultaneously [163,165]. Consequently, no effort was made to distinguish movements between groups from movements between areas [163].

More recently, studies have also considered dispersal behaviour in social (i.e. group-living) species [163]. As in solitary species, dispersal in territorial group-living species is associated with movements away from both familiar areas and conspecifics. However, in non-territorial social species dispersal may consist of movements away from a well-known area, from familiar conspecifics, or both. In some such species, the home ranges of different groups overlap, allowing animals to move between groups without leaving a familiar area (observed in gorillas (*Gorilla gorilla*) [166] and chimpanzees (*Pan troglodytes*) [167])[163]. Similarly, animals may also disperse in groups rather than individually, enabling animals to remain in a familiar social environment whilst leaving a well-known area. For example, whole social groups of vervet monkeys (*Cercopithecus aethiops*) may move away from degraded habitats or other groups in the same area [163,168]. Dispersal in groups (sometimes termed 'parallel dispersal') occurs infrequently in mammalian species compared to dispersal by individuals [143]. Where it does occur, individuals may disperse as whole social groups (e.g. *C. aethiops* [168]) or as subsets of a social groups (e.g. males in the Asian elephant, *Elephas maximus* [169]). As movement from familiar areas and familiar conspecifics is not necessarily coincident, some effort has been made to distinguish between these two types of dispersal in recent years. Where this distinction is made, movements from familiar areas are typically termed 'locational dispersal' and emigration away from known individuals is normally called 'social dispersal' (originally defined in [163]).

In some instances, authors use the term 'social dispersal' to instead refer to short-term movements outside of the social group for mating [169]. However, in most cases, authors do not classify these movements as dispersal, as they are reversed after a short period of time. Species that undergo only these 'temporary movements' are typically classified 'resident' or 'highly philopatric' (i.e. no dispersal of either sex occurs) [170]. Additionally, some authors refer to movements of individuals away from familiar areas as 'random locational dispersal' and movements of groups from known areas 'non-random locational dispersal' [169]. This occurs relatively infrequently, and authors usually define terms for clarity.

In most studies since the 1980s a broader definition of dispersal has been adopted [24,163,171,172]. Dispersal is now widely described as a permanent movement (i.e. one that is not reversed) away from a familiar range and/or from familiar individuals [24,163,171,172]. Throughout this thesis, I use this definition when referring to dispersal behaviour.

1.4.2.2. Distinctions due to the timing of dispersal

One of the earliest definitions of dispersal behaviour is that by Howard [164], which suggests that dispersal is any movement from a site of birth to an area in which an individual could reproduce. Whilst this was widely used in earlier studies, it does not fully describe dispersal behaviour. The definition by Howard [164] is appropriate for what is now termed 'natal dispersal'. Natal dispersal can be described as a permanent movement from the place of birth (i.e. the natal site) to a site in which an individual first breeds, or would have bred if they had survived to do so [24]. The timing of breeding relative to dispersal is critical in the definition of natal dispersal. Offspring may delay natal dispersal, such that juveniles from one breeding season remain in the natal area for prolonged periods without breeding (e.g. cooperative breeders [173]). As long as dispersal occurs before breeding, movements away from the area of birth can be considered natal dispersal. If reproduction occurs before emigration, then any dispersal would be 'breeding dispersal' (i.e. a permanent movement between groups in which individuals have reproduced). Animals may undergo breeding dispersal several times in their lifetimes, although it is relatively rare for species to undergo breeding dispersal at all [24,143,174–176]. In this thesis, I specifically consider natal dispersal.

1.4.2.3. Distinctions due to mating success

Most definitions of dispersal behaviour, including that by Howard [164] and the more recent definitions described above, do not specify that individuals must breed successfully after dispersal [24]. It is, however, generally assumed that animals will breed after dispersal, or would attempt to do so. If individuals do not produce young in the group to which they dispersed, this is termed 'gross dispersal'. If individuals are reproductively successful after dispersal, then the movement is instead classified as 'effective dispersal' [24].

1.4.2.4. Consequences of a poor definition

Several authors have cited the lack of a clear definition as one of the greatest hindrances to understanding dispersal behaviour (e.g. [142,145]). As the definition of dispersal varied, various different measures of dispersal behaviour were considered to be synonymous in many studies. Dispersal is typically measured according to the distance travelled or the

number of individuals departing [145]. The distance travelled is typically taken to be the straight line distance between sites [145]. The number of individuals dispersing is measured by recording the animals that depart from an area and/or group [145]. The margin of error in each of these measures can vary between studies depending on the monitoring methodology used, the size of the area and the corrections applied to the data [142,177] (discussed more in Section 1.4.3.). However, the raw data from studies considering one measure of dispersal behaviour is typically comparable with that from studies using the same measure.

As the two main measurements of dispersal (i.e. distance dispersed and the number of animals dispersing) were considered to be analogous, early comparisons of dispersal behaviour between species [24,141] included data obtained using both measures [142,145]. Dispersal is now increasingly recognised as a multi-stage process, rather than a simple movement away from an area and/or social group [142,145]. Specifically, dispersal is known to occur in three stages; emigration, exploration and settlement/immigration. Emigration is the movement of an animal out of the group and/or area. During the exploration stage, individuals search for a group and/or area in which to settle. At the final stage, referred to as either the settlement or immigration stage, individuals establish in an unfamiliar group and/or area [145,156]. Given this clearer definition of the dispersal process, it is obvious that the two measurement types consider different aspects of dispersal. By measuring the number of individuals dispersing, one is specifically studying the 'emigration' phase of the process. Studies that measure dispersal according to the numbers of individuals departing an area and/or group thus specifically assess what affects the decision of animals to emigrate. By contrast, studies that measure dispersal distance consider the 'exploration' stage. Such studies thus examine what affects decisions related to how far to move and where to settle [145]. As the two measures consider different aspects of dispersal, it is not possible to make direct comparisons of dispersal behaviour between species or populations unless a similar measure was used to obtain data. The results of studies that include data obtained using different measures of dispersal, like early comparative studies, are therefore confounded and unreliable.

Whilst the results of early comparative studies were confounded, they have been integral in developing dispersal theory [24,141]. This means that it is necessary to reassess widely accepted hypotheses initially proposed and tested in such studies. This would not have been a problem if dispersal behaviour had been clearly defined at an earlier stage. Further, because there was no recognition that different measures of dispersal considered separate

aspects of the behaviour, early studies rarely attempted to monitor dispersal in various ways. This contributed to the relatively limited level of data available on dispersal distance, as most studies considered only the number of animals dispersing (see Chapter 2 for further details).

1.4.3. Methods of monitoring dispersal behaviour

Although dispersal behaviour has generally been measured according to the number of animals disappearing or the distance moved, several methods have been used to obtain these data. In this section I discuss some of the methods used by researchers and some of the problems associated with each. I generally categorise the type of study used as either demographic (i.e. field studies that monitored the composition of populations) or genetic (i.e. studies completed using genetic data). Lastly, I will detail how the dispersal behaviour of species is classified in studies.

1.4.3.1. Demographic studies of dispersal

Genetic techniques have only recently been used to study dispersal [142,143,145], so most of what scientists currently understand about dispersal has come from demographic studies. Various methods have been employed in demographic studies. In general, the methodology consists of some form of observation of individuals within a population or populations.

1.4.3.1.1. Methods used in demographic studies

Many early studies of dispersal used trapping to monitor the movements of individuals. Mark-recapture methods were frequently used in early studies to infer the dispersal behaviour of species in various taxa, including insects (southern pine beetles (*Dendroctonus frontalis*) [178]). However, most studies considered birds (e.g. the bobolink (*Dolichonyx oryzivorus*) [179] and tree swallow (*Tachycineta bicolor*) [180]) or mammals (e.g. sea otters (*Enhydra lutris*) [181], plains vizcacha (*Lagostomus maximus*) [182] and banner-tailed kangaroo rats (*Dipodomys spectabilis*) [183]) (see [24] and [141] for further examples of early studies on mammalian and avian species). The technique continues to be used to the present day (e.g. recent studies on the rosalia longicorn beetle (*Rosalia alpina*) [184] and green turtle (*Chelonia mydas*) [185]). In mark-recapture studies, animals that are continually recaptured throughout their lifetime are typically considered to be resident in that area. Individuals that disappear from a site (i.e. are not captured after being sighted) are usually classified as 'dispersers' whilst those that appear within a site after a given period of time are considered 'immigrants'. The resolution of data obtained using mark-recapture methods can vary across studies according to several factors, including the length of the study season, the number of

seasons considered and the size of the study site. Variation in the level of data obtained can also occur both within studies. For example, Bollinger and Gavin [179] monitored dispersal of bobolinks at a site across several years. Land use and the accessibility of the area changed through time, so relatively less data could be obtained in later years. Despite these discrepancies, the technique remains a useful method to monitor dispersal behaviour, and can be used to measure dispersal distance and the number of animals dispersing, depending on the design of the study.

Other methods that have been, and continue to be, employed to monitor the movements of individuals include radiotelemetry/radio-tracking [186] and direct observations in the field [187]. Dahl and Willebrand [186] used radio-tracking to monitor movements in the mountain hare *Lepus timidus* for up to 3 years from birth. Depending on the exact methodology employed, radio-tracking can give greater levels of data on the movements of individuals compared to trapping; it is less reliant on the trappability of animals, and can allow movements to be tracked over a greater area [188]. Researchers can also monitor dispersal by simply watching and recording movements by individuals in a population (i.e. with direct observations). Direct observations are typically used for larger animals that are easily visible. For example, in Harcourt *et al*. [187] groups of mountain gorillas (*Gorilla beringei*) were observed over relatively long periods to monitor transfer of individuals between groups.

1.4.3.1.2. Issues with data from demographic studies

There are several limitations of demographic techniques which influence the quality of the data obtained [142]. Many of these limitations are related to restrictions in the level of resources available to conduct research. The level of such resources may limit the study area and/or the number of sites considered, the length of the study period and the intensity with which sites can be surveyed [142].

All demographic studies, regardless of the methodology used, are conducted within a finite area. Study sites can be relatively large (e.g. 13ha [182] and 22ha [179]), but they are unlikely to encompass the total area over which individual could disperse, particularly when considering larger or flying species. Demographic studies are therefore often limited in their ability to accurately measure dispersal distance [142]. Long distance movements have been detected in bats (e.g. Natterer's bats (*Myotis nattereri*) [188,189] and Daubenton's bats (*M. daubentonii*) [188]) using radio-tracking and mark-recapture techniques. However, this relies on capturing animals across multiple sites, and still does not necessarily provide an accurate

measure of dispersal distance. Thus, dispersal distance of species is often underestimated in demographic studies [142].

Demographic studies also usually overestimate the number of individuals that emigrate from a group [142]. In general, animals that disappear from a site (i.e. those which are not resighted or recaptured) are considered to have dispersed. Dispersal is not the only factor that can cause disappearance from a study area. Animals may instead disappear because the individual has died (due to age, disease, injury or predation). If the death is not recorded by researchers (e.g. with the location of a body), then this disappearance will be counted as a dispersal event. Alternatively, researchers could simply fail to relocate an individual that is present in the area, possibly as the individual begins to avoid traps [177]. The likelihood that a disappearance is misclassified as a dispersal event is correlated to the intensity of the study. For example, individuals are less likely to be relocated and deaths are less likely to be noticed if trapping or observations are conducted for short periods by few researchers during a study. In some cases, researchers have attempted to apply corrections to data from demographic studies to prevent overestimation of the numbers of animals dispersing, but the reliability of these is debated (discussed in [177]).

1.4.3.2. Genetic studies of dispersal

Over the last two decades, researchers have increasingly used genetic techniques, either alongside demographic methods [190] or in isolation [177], to study dispersal. It is hoped that the use of genetic techniques will increase both the number of dispersal studies that can be conducted and the reliability of data which can be obtained.

The quality of data from genetic studies is generally thought to be less likely to be negatively influenced by logistical problems [142]. This is primarily because long-term observations are not necessarily required to accurately monitor the general dispersal behaviour exhibited by different members of the population (often termed the 'dispersal pattern' of the population, see Section 1.4.4.1. for details). In genetic studies samples are taken from individuals within a population or populations to infer the spatial relationships of kin. These spatial relationships are used to make inferences about dispersal behaviour between groups or locations. Samples can be obtained in shorter time periods, and with less effort, than data could be obtained using demographic studies. It is therefore possible to assess dispersal behaviour of individuals over a greater area than in demographic studies, and studies require fewer researchers and people-hours in the field. This means that genetic studies can provide a more reliable indication of dispersal behaviour in a species than was possible with

previously available techniques (see e.g. [142,143,145,154,159,177,190,191] for further discussion on the relative advantages of using genetics to study dispersal behaviour).

Various techniques can be implemented to process data from genetic studies. Some genetic techniques are more sensitive than others. More sensitive techniques are relatively more able to detect dispersal over relatively short distances and fine-scale differences in dispersal behaviour between the sexes (i.e. sex-biases in dispersal) [159,177]. In relatively early genetic studies (particularly during the 1990's), traditional population genetic statistics (e.g. F-statistics (F_{ST})) were used to assess potential sex-biases in dispersal behaviour [177]. The relative power of different genetic methods was typically poorly understood [177]. One of the first studies to compare the sensitivity of different techniques was that by Mossman and Waser [177]. Mossman and Waser [177] examined sex biases in the dispersal behaviour of *Peromyscus leucopus* by comparing the spatial relationships of males and females. No significant sex biases were detected when using F_{ST} estimates, but a bias was evident when using an assignment index (AIc), which assesses the probability that an individual is born locally. This disparity in results using different methods was used to infer that assignment indices were more sensitive than F_{ST} indices. Thus, assignment indices are likely to be more appropriate to study dispersal in species where sex-biases are not very extreme (i.e. when there is little difference in the dispersal behaviour of males and females), as in *P. leucopus* [177].

In the late 1990s and early 2000s, most genetic tests on sex-biases in dispersal behaviour were based on assignment indices, F-statistics or heterozygosity [159]. These techniques rely on some genetic differentiation between population units, and measure immigration to these units and/or emigration away [159]. Perhaps unsurprisingly, some more recent techniques have relatively greater sensitivity. One example is individual-focussed mulitilocus spatial autocorrelation. This method does not necessitate that dispersal events are large enough to cause a sex bias in movement between population units. This method may be one of the most appropriate methods to assess dispersal behaviour over a relatively small scale (i.e. across tens of metres rather than kilometres) [159].

While genetic tests have advantages over demographic tests, it is clear that careful consideration is required to select the most appropriate technique for the species and populations under study. The selection of an inappropriate method could lead to erroneous results if subtle differences between the sexes or short dispersal movements are not detected. The sensitivity of the technique must be carefully balanced against the cost of

implementing that technique. More sensitive tests are often more expensive. Where funding is limited, researchers may choose to use less sensitive genetic tests to limit costs. This may mean that relatively subtle differences in dispersal behaviour are not detected. Restrictions in research funding could therefore limit data quality.

1.4.4. How to study dispersal behaviour; making comparisons across species

Many different methods have been used to study dispersal in species across several taxa [24,141–143]. Studies on individual species can be informative about what drives dispersal in that species. However, to understand what causes dispersal in general, it is necessary to make comparisons between species.

1.4.4.1. Classification of dispersal behaviour into dispersal patterns

In most studies of dispersal, regardless of the measure or technique used, the behaviour of males and females is compared to determine whether there is evidence of sex-biased dispersal (SBD) [24,141–143,145]. Dispersal behaviour is typically considered to be sex-biased if one sex dispersed significantly further or in greater numbers than the other. The bias may be in favour of males (male-biased dispersal (MBD)) or females (female-biased dispersal (FBD)). If the sexes disperse in relatively equal numbers or similar distances, then dispersal is classified as either unbiased or equal (ED), and if neither sex underwent dispersal then the species was considered highly philopatric (HP) [24,141–143,145]. These classifications are typically termed 'dispersal patterns'. I employ the above terminology throughout this thesis.

The dispersal pattern of the species influences population dynamics [24,141,142,192], so it is central in species survival, behaviour and ecology. It is the dispersal pattern of the species that is normally used to make inter-species comparisons of dispersal behaviour [24,141–143,145].

1.4.4.2. Attempting to understand the causes of dispersal using dispersal syndromes

As outlined above, dispersal is a key factor influencing several evolutionary and ecological processes [142,145,154,156,157]. Determining the dispersal pattern of a species, and why that pattern has been adopted, is crucial in understanding factors such as gene flow [160] and population dynamics [161], and in attempting to predict the longevity of that group [162]. Despite the importance of dispersal behaviour, the driving factors remain poorly understood [142,145]. The poor definition of dispersal and logistical difficulties inherent in studies are partly responsible, as they have limited the level of available data for tests. Until

relatively recently, there was no phylogenetic framework to enable large-scale comparisons across species, even for the best studied taxa (i.e. mammals and birds) [142,145]. Some comparative studies on dispersal behaviour have used relatively small phylogenetic trees to compare dispersal in closely related species (e.g. land snails [157], arvicoline rodents [193]). However, very few studies (although see [145]) have employed phylogenetic techniques to assess what may drive dispersal behaviour in larger species groups e.g. mammals and birds, in part due to the limited levels of data available [142,145]. Researchers now believe that phylogenetic studies, completed with large trees and greater amounts of data, will be key in elucidating the main drivers of dispersal behaviour [141].

To determine which factors may cause dispersal, it is necessary to determine whether the dispersal pattern of species coevolves with other traits, such as mating system [24,141–143,145]. Associations between dispersal behaviour and other traits are termed 'dispersal syndromes' [194]. The detection of dispersal syndromes can provide valuable insights as to what may cause a given aspect of dispersal behaviour to vary between species or populations of a species [157]. However, dispersal syndromes must be considered with care to prevent the over interpretation of results [195]. Indeed, coevolution may indicate causation (i.e. that the trait affects/is affected by dispersal behaviour) or simply correlation (i.e. the trait is related to a factor which influences or is influenced by dispersal behaviour [157]). Moreover, the dispersal behaviour of a species is likely the result of the interaction between multiple traits, so studies of dispersal syndromes provide a somewhat oversimplified view of the causes of dispersal. Careful further study of dispersal syndromes is thus necessary for determining what may influence dispersal behaviour [195].

Mating system is the factor most often associated with a given dispersal pattern (as seen in e.g. [24,141–143,145]). Indeed, this relationship has been a common focus of studies since Greenwood [24], who was the first to consider causes of dispersal in an evolutionary context for mammals and birds. Briefly, Greenwood [24] hypothesised that the adoption of mate-defence strategies by males should cause male-biased dispersal (MBD), whilst the adoption of resource-defence strategies by males should cause female-biased dispersal (FBD) [24,141,142,145]. The hypotheses outlined in that study (detailed and examined in Chapter 2) are widely accepted, but remain largely untested using modern phylogenetic techniques (although see [145]).

In recent years, dispersal behaviour has also been linked to several traits besides mating system. For example, Dahirel *et al.* [157] considered whether dispersal propensity (i.e. the

likelihood of dispersal) was determined by the level of habitat specialisation in European land snails. Species with high levels of habitat specialisation were found to be less likely to exhibit dispersal than species which were habitat generalists. Body size is also frequently linked to particular aspects of dispersal behaviour, particularly dispersal distance. Dispersal distances are known to be relatively high in larger species, as shown in land snails [157]. Qiu and Miyamoto [158] also showed that males dispersed further than females in avian species with male-biased sexual size dimorphism (i.e. where the males of a species are larger than females).

1.4.5. Ultimate and proximate causes of dispersal

An ultimate cause may be defined as the underlying reason for a behaviour, whilst a proximate cause can be considered the immediate trigger of that behaviour. Possible proximate and ultimate causes of dispersal have been studied widely since they were first contemplated almost forty years ago. Although the factors driving dispersal behaviour remain relatively poorly understood, several likely ultimate and proximate causes have been identified [142,145].

1.4.5.1. Ultimate causes of dispersal

To date, four ultimate causes of dispersal have been identified, although the relative importance of each is debated. Three of these, inbreeding avoidance, local mate competition and local resource competition, were first recognised by Greenwood [24]. The fourth, kin competition, has only been acknowledged within the last decade [142,196]. These factors were identified using data from mammals and birds [24,142], as these are the most well studied groups. However, these factors are thought to influence dispersal behaviour in all taxa [142,145].

1.4.5.1.1. Inbreeding avoidance

Inbreeding causes a reduction in genetic diversity within populations, which can lead to an increase in birth defects, immunodeficiency and reduced juvenile survival [197–200]. The chances of inbreeding are minimised if individuals can recognise and selectively avoid mating with relatives, or move away from known kin.

Natal groups include close kin, such as siblings and parents. Individuals rarely disperse with kin, so can typically reduce the chances of encountering, and thus potentially mating with, relatives after reaching sexual maturity by dispersing from the natal group [24,172,193,201–205]. Whilst natal dispersal can initially reduce the probability of inbreeding, the risk of

mating with relatives may increase in a breeding group over time if at least some of the offspring of an individual remains philopatric [206]. If the relative risk becomes sufficiently high, then inbreeding avoidance may drive breeding dispersal [204].

Inbreeding avoidance is thought to cause both natal and breeding dispersal in spotted hyenas, *Crocuta crocuta* [204]. Females remain philopatric and form kin groups, so offspring are related to all females. Females avoid inbreeding by refusing to mate with males born into the clan, so juvenile males must undergo natal dispersal in order to access mates. Females remain resident in the group, and avoid potential inbreeding with their sires by refusing to mate with any males that were resident in the clan before their birth. When the number of potential mates for a male reduces beyond a given level, males may attempt to increase their reproductive success by dispersing to another group in which access to mates will be greater [204].

Inbreeding avoidance is also thought to be an important driver of female-biased dispersal in at least some mammalian species. In mammals, the cost of reproduction is relatively greater in females [207]. Female mammals may therefore be more likely to attempt to minimise inbreeding. Inbreeding avoidance may be particularly important for females in non-monogamous species. In polygynous and promiscuous mammals, the reproductive fitness of males will show a greater level of skew than in females. Males of such species may breed with several females and sire young in multiple litters, whilst females invest their reproductive effort solely in to one litter. Reduced survival and fitness of offspring due to inbreeding will thus negatively impact the reproductive fitness of females more than that of males [208]. This could cause females to be more likely than males to disperse in plurally breeding mammals (discussed in e.g. [172,202]). When male tenure length is longer than the time required for females to reach sexual maturity, there is a risk of inbreeding between daughters and their sires [172,202,209]. Where this occurs, as in several primate species (e.g. chimpanzees (*Pan troglodytes*), mantled howler monkeys (*Alouatta palliata*) and gorillas (*Gorilla gorilla*) [202]), inbreeding avoidance is expected to be associated with a female-biased dispersal pattern (see [202] for a detailed discussion).

Although inbreeding avoidance may drive dispersal in some species, individuals may avoid inbreeding by refusing to mate with likely kin in the natal group. 'True' kin recognition is possible using polymorphic markers including the major histocompatibility complex (MHC) and murine urinary proteins (MUPs) [210]. Individuals could make use of these markers to avoid mating with kin. Animals may also be able to selectively mate with unrelated individuals

without dispersal by mating with individuals outside the natal group during temporary movements. This strategy enables individuals to gain benefits associated with remaining with their kin whilst avoiding the negative effects associated with inbreeding. This means of inbreeding avoidance is rare in mammals, but has been adopted by several cetacean species including bottlenose whales (*Hyperoodon ampullatus*) [211], pilot whales (*Globicephala melas*) [212] and killer whales [170,213].

Dispersal is a costly behaviour. The fact that inbreeding avoidance can be achieved in the absence of dispersal has caused the relative importance of inbreeding avoidance as a driver of dispersal to be debated in recent years [142]. Nevertheless, for at least some mammalian species it is recognised as a primary determinant of natal and breeding dispersal patterns [202,204].

1.4.5.1.2. Local mate competition

When access to one sex is relatively limited, competition for mates (i.e. mate competition) is likely to occur [24]. The operational sex ratio (OSR) of a population is the number of sexually active males compared to the number of sexually mature females. In populations with a skewed OSR, the more abundant sex will experience local mate competition [24]. An equal OSR does not necessarily ensure that mate competition will not occur, as one sex can restrict access to the other. For example, in lions (*Panthera leo*) dominant males will prevent subordinate males within the same coalition from mating with females. Equally, members of a coalition will compete vigorously with other males to ensure sole mating access to a group of females (discussed in [19]). Males compete for access to mates continually throughout their lives [19], so access to mates could drive both natal and breeding dispersal in male lions. Local mate competition could theoretically drive dispersal in either sex [24]. However, in mammals, males are more likely than females to experience high levels of mate competition [8,24,141,142,145]. As such, local mate competition is more likely to drive the dispersal of males [24].

1.4.5.1.3. Local resource competition

Resource requirements differ between individuals and over time, but are likely to be more similar in members of the same sex and at the same reproductive stage. Some resources are required solely by one sex. For example, in mammals, maternal care is ubiquitous [214] whilst paternal care is rare [215,216]. Females are thus usually solely responsible for securing access to resources that are necessary for successful reproduction, including nest sites [5]. When only one sex requires a given resource, competition for that resource will only occur intra-

sexually. If a resource is required by both sexes, but one sex or at least some members of one sex requires that resource at greater levels, then competition for that resource will act primarily intra-sexually. The level at which a resource is required may vary according to age, sex or reproductive state. For example, lactation is energetically costly [217], so the energetic and nutrient needs of lactating females will be greater than for other members of the population [214]. All members of the population will compete for food, but lactating females are expected to compete most vigorously with each other.

Many resources are necessary for survival and successful reproduction. Individuals precluded from these resources at one location or in one population may disperse in order to gain access. If competition for a resource occurs primarily or solely within one sex, then that sex is more likely to disperse. Moreover, if individuals are forced to disperse by other members of the same population, then it is most likely to be of individuals of the same sex [5]. The level of competition, and thus the likelihood of dispersal, will correlate with the availability of that resource. Dispersal due to local resource competition, as any of the ultimate causes of dispersal, can thus be active (i.e. individuals are driven to disperse by other) or passive (i.e. individuals decide to disperse of their own volition) [24].

There is evidence that local resource competition may drive both natal and breeding dispersal in at least some species [5]. Female Siberian flying squirrels (*Pteromys volans*) must secure a territory that contains nest sites in order to breed successfully. Suitable territories are scarce, and competition between females is high. Older females are likely to outcompete juveniles, so juveniles may be expected to be more likely to disperse. Hanski and Selonen [5] found that although juvenile females dispersed from the natal site, this typically occurred after the emigration of the mother, indicating that juveniles were not forced to disperse. They suggested that adult females may emigrate so that one female offspring may 'inherit' the breeding site. Any other female juveniles were expected to disperse after one has dominated access to the resource. As such, competition for resources can be said to cause natal and breeding dispersal in *P. volans* [5].

In mammalian species, the energetic costs of reproduction are generally greater for females, particularly as maternal care is ubiquitous whilst paternal care is rare [146,147]. Mammalian females typically compete for resources other than mates [24,141,142]. Thus, local resource competition is expected to drive the dispersal of females rather than males in mammalian species [24]. However, local resource competition may also drive the dispersal of males. In species with paternal care, members of both sexes could experience similar levels of resource

competition because males may attempt to secure the same resources as females. In such species, local resource competition may drive dispersal in both males and females [24].

1.4.5.1.4. Kin competition

Individuals can maximise lifetime reproductive success by gaining as many direct and indirect fitness benefits as possible [218,219]. This means that individuals should adopt a strategy which allows them to produce as many offspring as possible and, where possible, should avoid limiting the reproductive success of their kin [218,219]. Individuals may limit the reproductive success of kin by competing with them and thus restricting or prohibiting access to resources, including mates, required for successful reproduction. Kin competition (i.e. competition between kin) for such resources is most likely to occur when relatives remain together after sexual maturity. If individuals disperse as groups or remain philopatric then the risk of kin competition is high, but it is low if animals disperse independently of relatives (discussed further in Chapter 3). Thus, kin competition and dispersal are related, and recently it has been recognised as a possible ultimate driver of dispersal [196].

Inbreeding avoidance, local resource competition and local mate competition have received a considerable amount of attention as drivers of dispersal. By contrast, kin competition has received relatively little attention [142,196]. Perhaps the earliest theoretical evidence that kin competition could drive dispersal is that from Hamilton and May [220]. In that study, a game-theoretical approach was used to show that kin competition could cause the evolution of dispersal. Several studies have since found empirical and theoretical evidence in support of those early results [221–227]. Most recently, Brom *et al*. [196] used a modelling approach to show that kin competition could be a primary cause of sex-biased dispersal patterns across taxa. Their model focussed specifically on intra-generational kin competition, rather than parent-offspring conflict or kin cooperation, both of which have also been implicated in the evolution of dispersal [223,228]. This latest evidence is perhaps the best theoretical evidence to date that kin competition could be an ultimate cause of dispersal.

As with all of the ultimate causes of dispersal, kin competition is likely to drive dispersal in the sex which is most likely to be negatively impacted. Individuals are most likely to be around relatives in their natal groups, so kin competition can drive natal dispersal. Kin competition could also cause breeding dispersal if kin remain together after reproduction. Further study will be required to determine how frequently kin competition is likely to cause either natal or breeding dispersal.

1.4.5.1.5. Potential for interactions between the ultimate causes of dispersal

The ultimate causes of dispersal are often discussed separately, as above, but they are unlikely to act in isolation. In most cases, as with the examples presented here, the dispersal pattern of a species is explained with respect to the factor which is most likely to have caused that dispersal pattern. However, other factors may also influence the dispersal behaviour of the species. In reality, the dispersal pattern of any species or population will typically result from the interaction of several factors [142,145].

For example, inbreeding avoidance is likely to be the main cause of male-biased dispersal in *C. crocuta*; if females did not reject males to avoid inbreeding, males may not disperse [204]. The costs of reproduction are greater for mammalian females than males [207], so females are more likely to actively try to avoid inbreeding [172,209]. In *C. crocuta*, the rejection of potentially related males indicates that females attempt to avoid inbreeding, but there is no evidence that males would not mate with related females if possible. Males may disperse solely because the inbreeding avoidance behaviour of females limits mate availability. Inbreeding avoidance and local mate competition could therefore be said to interact to cause male-biased dispersal in *C. crocuta*.

Ideally, studies should consider any potential interactions when studying dispersal so that the behaviour can be more fully understood. This could also reduce the possibility of misinterpreting which factors most influence dispersal [195].

1.4.5.2. Proximate causes of dispersal behaviour

Researchers deduced which factors were likely to be ultimate causes of dispersal by comparing data across species [24,141,142]. Modelling approaches have since been used to validate and reconfirm the deductions of earlier studies (see e.g. [145,196] and references therein). By contrast, proximate causes of dispersal have generally been studied in individual species (e.g. [154]), although recent studies have also considered small groups of closely related species (e.g. [157]).

Many proximate causes of dispersal have been identified, and I will not attempt to describe each of them here (see [156–158,177] for more examples than those presented). Researchers have attempted to categorise proximate causes to more easily reference them, but the classifications used vary. Clobert *et al*. [229] classified dispersal caused by environmental cues, including seasonal cues of when to emigrate [177], as 'condition dependent dispersal'. If dispersal behaviour was instead related to the phenotype, including

relative boldness [230,231], this was termed 'phenotype dependent dispersal'. In this system, 'condition dependent dispersal' refers to the condition of the environment, not the body condition of the individual. Rémy *et al*. [232] instead classified proximate causes of dispersal as 'extrinsic' or 'intrinsic'. Extrinsic factors include anything which could be considered an external cue for dispersal (i.e. one given by the environment of that animal). Extrinsic factors include population density, population sex ratio and the nature and impact of social interactions (reviewed in [233,234]). Intrinsic factors include those which act internally (i.e. features of individuals which may cause dispersal). Some examples of intrinsic factors are body condition [235], sex [24] and developmental and parental effects [236–240] (discussed in [156]).

Each of the proximate causes of dispersal can be related to an ultimate cause. For example, the proximate trigger for dispersal could be low resource availability [177]; ultimately though, dispersal may occur because local resource competition is high. Proximate causes, as with ultimate causes, are likely to interact to influence dispersal behaviour. For example, resource availability may be low because population density is high and/or habitat quality is low, both of which would increase competition for resources. It is important that proximate factors are studied with care to ensure that causation is not assumed from simple correlation [195].

As with the ultimate causes of dispersal, the proximate causes of dispersal remain largely understudied [157] and many proximate causes may remain unidentified. Understanding proximate causes is likely to provide a greater insight as to what ultimately drives dispersal. Some factors may be relatively more important than others, and research on such factors should be prioritised. For example, Dahirel *et al*. [157] recently stated that the drivers of dispersal would be better understood if factors influencing the propensity and capacity for dispersal are studied in greater depth.

1.4.6. Costs and benefits of dispersal

Animals typically disperse when the costs of remaining in an area are likely to be greater than those associated with leaving that area. Often, the costs of remaining in an area are greater than dispersing when an individual is likely to gain greater reproductive success by dispersing. If resource and/or mate competition becomes sufficiently high individuals may not be able to secure the resources, including mates, required for successful reproduction. An individual that is unable to reproduce in one area due to resource limitation may disperse in an attempt

to gain the resources needed to reproduce in a different location and/or social group. Other benefits of dispersal include evasion of both kin competition and inbreeding.

There are many costs associated with dispersing from an area. Bonte *et al*. [241] categorised these into four main types: energy, risk, time and opportunity. Perhaps the most obvious costs of dispersal are energetic. Individuals typically undergo long-distance movements during dispersal, which are extremely energetically costly. Other energetic costs include those associated with the development of phenotypic traits which are specialised for dispersal, such as wings in the alder aphid (*Pterocallis alni*) [242]. During dispersal, animals are at greater risk of predation, injury and settlement in unfavourable habitat. The risk is relatively greater during dispersal because animals are not familiar with the area over which they travel. Unfamiliarity with an area can increase predation risk because animals are less likely to be able to evade predators when they are not aware of possible shelter in the habitat [243,244]. The length of time required to disperse is likely to vary widely dependent on the capacity of an individual for long-distance movements and the proximity of a suitable habitat/group. The time invested in dispersing cannot be used for activities such as reproduction. Dispersal may also lead to losses in opportunities. For example, if an animal emigrates from one social group in to another, it typically obtains a lower rank in the second group. By dispersing, the animal loses the opportunity to rise in the ranks and potentially reproduce in the natal group (see [241] for a more detailed discussion).

Given the high costs and various potential benefits of dispersal, many researchers have adopted the view that dispersal is a behaviour which is beneficial to those which undertake it [23]. Anderson [245] termed that hypothesis 'the emigrant fitness hypothesis' (EFH). The EFH posits that dispersal evolved because it confers an advantage to dispersers, and that dispersal is controlled by their genes. Anderson [245] opposed the emigrant fitness hypothesis, at least for rodents, and instead favoured an alternative, which he named 'the resident fitness hypothesis' (RFH). The resident fitness hypothesis proposes that, rather than being beneficial to the emigrating individual(s), dispersal is beneficial to and driven by those which remain resident within a group (typically the parents of dispersers).

In most cases, dispersal is thought to be advantageous to emigrants, so the EFH is generally favoured. However, there is evidence that parents may benefit from the dispersal of offspring. If offspring remain philopatric, competition in the home range of the parent (i.e. the natal home range) will be increased as more animals will attempt to access the same resources. Offspring philopatry will also cause a relative increase in the risk of inbreeding.

Thus, by ensuring the dispersal of young, residents ensure that the level of competition (including kin competition) does not increase, prevent the over exploitation of resources [242] and keep the risk of inbreeding relatively low (discussed in [245]). There is less direct evidence for the RFH compared to the EFH, although in some of the species tested, there is evidence for both hypotheses. Gliwicz [23] evaluated benefits to both dispersers and residents in bank voles (*Myodes glareolus*), and found evidence that offspring dispersal benefited both groups (discussed further in Section 1.6.1.2.).

1.5. Maternal effects

As explained previously, in this thesis I explore reproductive competition, dispersal behaviour and the associations between them. I also consider the impact of maternal effects on traits related to both dispersal behaviour and reproductive competition in mammals. I begin this section by defining maternal effects before discussing the study, impact and relative importance of maternal effects.

1.5.1. The definition of maternal effects

Maternal effects have historically been defined as any effect of the maternal environment on offspring phenotype [246]. One more recent definition considered a maternal effect to be any impact of the genotype/phenotype of the mother on offspring phenotype [247]. This latter definition excludes any changes in offspring phenotype related to the mitochondria or cytoplasmic inheritance [247]. In this thesis, as in many relatively recent studies [248], I consider a maternal effect to be any influence of the mother on the phenotype of offspring which is not determined by offspring genotype.

1.5.2. The study and impact of maternal effects

Maternal effects were first described almost 70 years ago [246]. In early studies, maternal effects were considered to be relatively unimportant, except as potential confounding factors on traits of interest [249]. In early studies maternal effects were thought to be exclusively maladaptive [248]. In many cases, the negative impacts of maternal effects were thought to result because the mother was relatively malnourished during pregnancy and/or whilst providing maternal care [248]. This caused offspring to be less competitively able, ultimately resulting in reduced offspring lifetime success/fitness [248].

It is now widely acknowledged that, whilst maternal effects can have a negative impact, they may also act to improve offspring fitness [247,248,250]. Indeed, maternal effects are now

widely recognised as highly adaptive mechanisms by which mothers can influence offspring phenotype to maximise offspring fitness in the local environment [249,251–255].

Maternal effects can act on a wide variety of traits, including aspects of reproductive investment and dispersal behaviour (discussed further in Chapters 4 and 5, respectively). Consequently, maternal effects have been implicated in a variety of processes including population dynamics [253,255–258], niche construction [259], response to selection pressures [260–262] and the evolution of life history traits [255,258,263–273]. Maternal effects have been observed in many taxa including mammals [253–255,260,263–265,267,268,270–275], birds [266,276], fish [252,257], plants [277], reptiles [261] and insects [81,278]. As such, maternal effects can have massive implications across many industries and fields of study. Examples include commercial farming and fishing [257,268,275,277], medicine [247,269,273,279] and species conservation [247,260,261].

Any impact of maternal effects may persist through generations [253]. This, together with the wide variety of traits and processes which can be impacted, means that it is important that the action of maternal effects and the consequences for offspring are well understood. Despite this, maternal remain largely understudied [246,247].

1.5.3. Relative importance of maternal effects and other factors affecting offspring phenotype

Maternal effects are not the only factors which can influence offspring phenotype in a manner which is not determined by offspring genotype. Indeed, offspring phenotype may also be altered due to paternal effects and, in species with external fertilisation and/or offspring gestation, by environmental conditions [254,278,280,281]. Paternal effects, like maternal effects, are a type of parental effect [246,247] (i.e. an influence of a parent on offspring phenotype which does not result from offspring genotype [248]). Paternal effects are parental effects caused by the father, rather than the mother. Environmental effects are any persistent changes in offspring phenotype which are caused by environmental conditions. Temperature dependent sex determination could be considered an example of an environmental effect [282].

Maternal effects are typically considered to be relatively important compared to paternal or environmental effects, particularly in viviparous species or species with post-natal maternal care. As gestation occurs internally in viviparous species, any aspect of offspring development before birth could potentially be influenced by the mother [251]. Although gestation is internal, paternal effects can act during development in viviparous species. For

example, sires may influence offspring phenotype by altering gametes used to fertilise eggs [280]. However, paternal effects are detected relatively rarely, and when present they typically influence offspring phenotype to a lesser extent, so maternal effects are considered to be relatively more important [281]. Offspring phenotype may also be influenced by environmental, paternal and/or maternal effects post-partum. Maternal effects are typically considered to be the most important if the species exhibits uniparental care either primarily or exclusively by the mother. In mammals, maternal care is ubiquitous (primarily due to lactation), whilst paternal care is relatively rare and can be limited when it does exist (see e.g. [146]). Maternal effects are therefore likely to have a greater influence on offspring phenotype than paternal effects post-partum in mammals, particularly as chemicals transferred during lactation may influence offspring phenotype [263,267].

1.6. The bank vole (*Myodes glareolus*)

In this thesis I use the bank vole (*Myodes glareolus*) to study some aspects of reproductive competition and dispersal behaviour. The bank vole is regularly used as a model for the evolution of life-history traits, so the results of the studies described herein can be applied to other, similar species.

1.6.1. A brief account of the general ecology

The bank vole (*M. glareolus,* formerly *Clethrionomys glareolus*) is a generalist consumer rodent which is a common resident across Europe, and the rest of the Palearctic ecozone [283–285]. A picture of a bank vole is provided in Figure 1.1 for reference. Bank voles have a relatively short life expectancy, and individuals rarely overwinter twice [286,287]. Bank vole populations are characterised by seasonal and multi-annual fluctuations in density [288]. The extent of these fluctuations is likely to vary across the species' range. One study in Pallasjärvi (Finnish Lapland) showed that density could vary by a factor of 10 to 100 within 4 years [289]. Another study in the Ardennes (southeast Belgium) , showed variation between 1 and 70 voles per hectare [290,291]. Several factors have been implicated as the cause of these fluctuations. Most often, the cyclic nature of bank vole populations has been attributed to reproductive traits [285]. Offspring born in high density populations exhibit reproductive suppression throughout their lives, resulting in a reduction in population density [292]. Population density may then increase sharply at a later date, as bank voles can produce young rapidly [285].

Both males and female bank voles can exhibit territoriality during the breeding season [23,293–295], but not whilst overwintering [296]. There is some evidence that home ranges

of individuals vary according to a wide variety of factors. Korn [297] found that variation in home range size was not significantly related to body size and reproductive stage. This suggests that the energy requirements of individuals are not a major determinant of home range size [297]. By comparison, there is evidence that bank voles and other vole species have relatively small home ranges where cover is abundant [297–299], population density is high [297,300,301] and/or the availability of food resources is increased [297,299]. Home range size also varies according to sex, with males having generally larger ranges [302,303].

Figure 1.1. Photograph of an adult bank vole, sex unknown. Picture used with permission from Roger Butterfield © 2007.

1.6.1.1. Reproductive characteristics and reproductive competition

The length of the bank vole breeding season varies between years and between populations within years, but it typically extends between spring and autumn (e.g. April to September in Crabapple Island, Poland [303]). Fluctuations in breeding season length do not appear to influence population dynamics [304,305], and may instead relate to environmental

conditions and habitat quality. Females produce 3-4 litters per year in the wild [23,285], each containing between 2 and 10 pups [283]. The average litter size of a population varies throughout the breeding season [306] and according to resource availability [307]. Ovulation in females is induced by mating [308,309], so the production of litters is not necessarily synchronous within a population. Males and females can become sexually mature at ~2 and ~1.5 months, respectively [306], although reproductive suppression may occur, causing infertility or delayed maturation [292,310].

During the reproductive season, the territories of breeding males and females overlap intersexually [23,293,294,311]. This enables both males and females to mate with multiple partners [23,311], resulting in a promiscuous mating system. Females exhibit a high degree of polyandry, and many litters are multiply sired [312]. All males are likely to encounter sperm competition. Accordingly, males have evolved large testes relative to their body mass [80]. There is evidence that the number of males which mate with a particular female varies according to population density, with females more likely to mate multiply in high density conditions [313]. Male bank voles exhibit a dominance hierarchy [314], consisting of dominant and subordinate individuals. When population density is low, dominant males are able to secure primary access to females within their territory, and subordinate males are excluded from breeding [313]. By contrast, when population density is high, subordinate males are able to mate with females [313]. Females are likely to mate with more males in high density populations both because there are more males present and because individual males are less able to monopolise access to a given female. The level of post-copulatory competition between males is thus likely to be greater in high density populations. There is some evidence that males can vary investment in post-copulatory competition according to population density. Indeed, young males that are exposed to cues that indicate a higher level of male competitors develop seminal vesicles which are larger both absolutely and relative to body size [119].

The reproductive success of female bank voles is dependent on the ability to secure a territory [301,315]. In some studies, females are considered to be highly territorial throughout the breeding season, with individuals excluding all other females from that range [315]. However, there is evidence that females may only be highly aggressive and territorial in late-stage pregnancy [311], and that they frequently share territories with related same-sex conspecifics [311]. Indeed, the reproductive success of females is relatively increased in so called ‘female kin groups’, in which females in neighbouring territories are related [311]. The territories of males may also overlap, leading to aggression between males [302].

1.6.1.2. Dispersal behaviour

In bank voles, the natal dispersal pattern is considered to be male-biased [316]. Most, if not all, males disperse from the natal site, whilst the propensity of females to disperse is thought to be relatively low [23]. The dispersal behaviour of female bank voles remains relatively poorly understood [23]. However, both males and females are thought to disperse in order to find a territory to enable reproduction [317,318]. Natal dispersal in most mammals is expected to occur at or around the timing of sexual maturity, and individuals tend to breed for the first time after settlement in a new area and/or social group ([24]; Section 1.4.2.2.). In bank voles, there is some evidence that males are sexually mature when they disperse, but that dispersing females are not [316]. Members of both sexes can travel over large distances when emigrating [319], although there is some evidence that dispersal distance is greater in males [316].

The natal dispersal behaviour of juveniles of both sexes may vary according to the relative timing of birth in the reproductive season [23,316]. Males emigrate in higher numbers in spring than in autumn [316]. Females which are born in the first litter of the year (typically in spring, during the second month of the breeding season) are more likely to disperse than those born in subsequent litters in the same year [23]. Gliwicz [23] studied dispersal in bank voles, particularly in females. Early in the breeding season, the optimal habitat was densely occupied with overwintered individuals. Females in the first litter of the year dispersed to the suboptimal habitat in order to establish territories and reproduce. As the life expectancy of the bank vole is low, the mortality rate of overwintered individuals increased throughout the year. When females born in the second litter of the year reached the age of first possible sexual maturity (~1.5 months), territories were available in the optimal habitat as previous residents had died. However, the number of newly vacant territories was insufficient for each female born in later (i.e. the second and third) litters of the year to attain separate territories. Females which did not disperse remained philopatric until the next breeding season. Thus, although some females from the later litters could disperse and establish territories, more territories were available for females born in the first litter, so relatively more emigrated from the natal area [23].

As bank voles rarely overwinter twice [23,285–287], individuals are likely to survive a maximum of two breeding seasons; that in which they were born and that in the year after birth. Females that disperse from the natal area are more likely to mature and reproduce in the year of their birth, and may thus have greater levels of reproductive success than those

that do not [23]. This is clearly evidence in support of the emigrant fitness hypothesis (EFH) in bank vole females [23]. There is, however, also evidence for the resident fitness hypothesis (RFH). When young remain philopatric, the resources available in the territory of the female must be shared. If young disperse then fewer individuals share resources, so mothers may benefit from the dispersal of young [23]. Gliwicz [23] also suggested that the dispersal of females from the first litter also benefits young in the second litter; females from the second litter only matured after the dispersal of females from the first litter. This, in turn, benefits mothers as it maximises the potential lifetime reproductive success of daughters. Gliwicz [23] concluded that both residents and emigrants benefitted from the natal dispersal of bank vole females. This, together with evidence from the study by Prévot-Julliard *et al*. [320], suggests that the delayed sexual maturity and relatively low dispersal rates of females are due to social constraints and limited availability of territories, not because it confers some benefit. Males may be less likely to be affected by the number of vacant territories, as mature males show relatively more territorial overlap than adult females [295,313].

Breeding dispersal has been observed in adult bank voles. Early studies suggested that many individuals exhibited breeding dispersal (discussed in [318]). More recently, a study by Gliwicz and Ims [318] suggested that breeding dispersal was both less common and less adaptive than previously expected. As in most species, breeding dispersal in bank voles is understudied compared to natal dispersal.

1.7. Thesis overview

This thesis seeks to enable greater understanding of the causes and consequences of dispersal in mammals, and how these may interact with reproductive competition. In the first half of the thesis, I present the results of comparative studies, in which phylogenetic analyses are used to test hypothesised associations between dispersal behaviour and various forms of reproductive competition across mammalian species. In the second half, I present the results of experimental studies in which I used bank voles (*Myodes glareolus*) as a model to explore potential maternal effects on dispersal behaviour and reproductive traits linked to intrasexual competition.

In my first data chapter, I consider whether the mating system of mammals influences the dispersal pattern exhibited (**Chapter 2**). I also test how the relatedness of breeding males, as determined by their natal dispersal behaviour, impacts investment in pre- and post-copulatory competition (**Chapter 3**). I then consider how maternal effects caused by variation

in population density can influence life history traits (**Chapter 4**) and dispersal behaviour (**Chapter 5**) in bank voles.

I end the main body of the thesis with a general discussion of the results and conclusions of the studies completed. In that final chapter, I reflect on the broader implications of my research in mammals and other vertebrates (**Chapter 6**).

Chapter 2: Do mating systems influence natal dispersal behaviour in mammals?

Abstract

Potential relationships between mating system and dispersal patterns have been discussed for four decades, but remain poorly understood. Several evolutionary relationships between these traits have been proposed for mammalian species, with mating system thought to cause specific dispersal patterns. Male-biased dispersal patterns have been linked to the adoption of mate-defence strategies by males, which is typically observed in polygynous and promiscuous species. Monogamy has been associated with female-biased dispersal where males adopt resource-defence strategies to attract mates. However, other studies have proposed that dispersal behaviour should be unbiased (i.e. equal) in monogamous species, perhaps particularly where males provide depreciable forms of parental care including offspring provisioning. Here I investigated each of these widely accepted hypotheses using a comparative approach. There was no evidence to support any of the proposed relationships between mating system and dispersal behaviour, although there was evidence for coevolution between monogamy and equal dispersal. Taken together, the results indicate that mating system is a poor predictor of dispersal behaviour in mammalian species. Future studies should instead attempt to evaluate ultimate causes of dispersal, including levels of local mate and resource competition, to predict and explain dispersal patterns in mammalian species.

2.1. Introduction

2.1.1. The causes and consequences of natal dispersal behaviour

Natal dispersal has recently been defined as a permanent movement from the place and/or social group of birth (i.e. natal area and/or group) to the area/group in which breeding first occurs (i.e. the breeding group/site) ([24,141,142,145,156,158]; see Section 1.4.2. for further details on definitions of dispersal). The natal dispersal behaviour of individuals determines the spatial distribution of animals, and thus the genetic structure of populations [142,145]. Natal dispersal behaviour therefore fundamentally influences a wide range of ecological and evolutionary processes [142,145,154,156,157]. Indeed, dispersal is key to understanding, among other things, gene flow [160], population dynamics [161] and capacity to respond to environmental change [162].

Given the importance of natal dispersal, it is unsurprising that the potential causes and consequences of the behaviour have been the focus of many studies since the 1970s [24,142]. Several factors have been proposed as ultimate or proximate causes of dispersal [142,145]. Greenwood [24] was the first to consider evidence for potential drivers of natal dispersal behaviour in an evolutionary context. In this widely cited contribution, Greenwood [24] proposed that dispersal was ultimately driven by local resource competition, inbreeding avoidance and local mate competition. More recently, kin competition has also been recognised as a probable ultimate cause of dispersal behaviour [142,196,321]. Studies on individual species and species groups have led to the identification of several proximate causes of dispersal behaviour including physiological processes, seasonal cues and parental effects [322].

The ultimate and proximate drivers of dispersal may act differently on members of each sex, resulting in sex biases in dispersal behaviour [24,141]. For example, the reproductive strategies adopted by males and females may mean that one sex is more likely to experience mate competition, or more likely to be negatively affected by inbreeding avoidance. This could cause dispersal propensity and/or dispersal distance to be relatively greater in one sex [24,141–143,145]. The dispersal pattern of a population of a species describes the relative dispersal behaviour of each sex within that population [24,143,145,172,193,201,203,205]. The natal dispersal pattern may describe either the frequency of individuals dispersing (i.e. dispersal propensity) or the distance dispersed from the natal area and/or group [24,141–143,145]. Populations may adopt one of four dispersal patterns: male-biased dispersal (MBD), female-biased dispersal (FBD), equal dispersal (ED) and high philopatry (HP). Dispersal is considered to be male- or female-biased (i.e. sex-biased) if males or females, respectively, are significantly more likely to disperse or to disperse further than individuals of the other sex. In species with ED, both sexes are equally likely to disperse or to disperse similar distances. If neither sex disperses, or if dispersal is rare, then the species is said to exhibit high philopatry (i.e. individuals never permanently leave the natal site) ([24,141]; more details available in Sections 1.4.2. and 1.4.4.1.).

In order to gain an understanding of the dispersal behaviour of a population, it is necessary to investigate what causes a given dispersal pattern and the subsequent consequences. However, despite the importance of dispersal behaviour, and the consequent high level of interest in this area, the causes and consequences of dispersal behaviour remain poorly understood.

2.1.2. Why are the drivers of natal dispersal behaviour so poorly understood?

Historically, studies of the causes of dispersal have been hindered by three primary factors; a poor definition of dispersal, logistical difficulties involved in studying dispersal and the absence of a phylogenetic framework for comparative studies across species.

2.1.2.1. Poor definition of dispersal behaviour

In many early studies, authors defined dispersal as a movement from a familiar area to an unfamiliar range, so dispersal was measured according to the number of individuals that left an area and/or group (as in Dobson [141]). In other studies, dispersal behaviour was defined according to the distance moved, so dispersal was quantified by measuring the distance travelled between sites (as in Greenwood [24]) [142,145].

Dispersal is now recognised as a multi-stage process comprising of emigration (movement out of group and/or area), transition (movement between groups and/or areas) and immigration (settlement into a new group and/or area) [145]. Given this definition of dispersal behaviour, it is clear that the two measures of dispersal behaviour consider different stages of the process [145]. Specifically, measures of the number of animals leaving a site provide insights about what causes animals to emigrate, whilst measures of dispersal distance can be used to investigate which factors influence how far individuals move or where they settle.

In early studies there was no distinction between measures of dispersal propensity and distance, so they were considered together in early comparative studies. Thus, the results of early comparative studies (e.g. [141]), which were fundamental in the development of dispersal theory, are confounded [142,145].

2.1.2.2. Logistical difficulties and measurement error in dispersal studies

Genetic techniques have only recently been applied to the study of dispersal behaviour, so most studies to date have employed relatively research intensive methods including trapping, radio-tracking and direct observation [142]. The logistical difficulties inherent in these methods have limited the number of studies on dispersal behaviour. Moreover, the data obtained using these approaches often contains measurement error, as studies occur in a finite area and it is not easily possible to effectively monitor all animals in a population [142,145]. This typically leads to an overestimation of the number of animals that disperse, and an underestimation of the dispersal distances of individuals [177]. Mathematical

corrections are often applied to the data from demographic studies in an attempt to account for measurement error, but the reliability of such corrections is uncertain [177].

It is hoped that the application of genetic techniques to studies of dispersal behaviour will increase the frequency of studies, and enable the collection of more accurate and reliable data. This should, in turn, facilitate more comparative studies to determine the causes of dispersal [142].

2.1.2.3. The lack of a phylogenetic framework

Cross-species comparative studies are crucial for understanding what drives dispersal behaviour [142,145]. To gain reliable results from such studies, it is crucial that the evolutionary relatedness of species is accounted for [323,324]. Until relatively recently though, phylogenetic trees, particularly those which contain several species from across orders within a class, have not been available [141,145]. Early comparative studies considering dispersal behaviour, including those in highly influential papers by Dobson [141] and Greenwood [24], were therefore completed without phylogenetic correction. To date, relatively few phylogenetic studies of dispersal have been completed. Those that have been conducted are based on relatively few species, due in part to limited data availability [142,145].

2.1.3. Studying the causes of dispersal behaviour

To identify potential proximate and ultimate causes of dispersal, it is necessary to investigate which factors coevolve/covary with an aspect of dispersal behaviour (e.g. dispersal pattern) [24,141,142,145,155–157,194,229]. However, any relationships detected must be considered carefully to prevent over interpretation [194,195,229]. Indeed, covariation between traits does not necessarily imply causation [195], and dispersal behaviour may covary with a trait/factor which neither affects, or is affected by, dispersal behaviour [195]. Studies that consider the relationship between dispersal behaviour and one other trait are necessarily oversimplifications, as dispersal behaviour is likely influenced by the interactions of several factors [142,194,195]. Nevertheless, such studies can be highly informative in identifying potential causes and consequences of dispersal behaviour [142].

2*.1.4. Proposed associations between mating strategy and dispersal pattern*

The trait that has most often been linked to dispersal behaviour is mating system [24,141–143,145]. Potential relationships between dispersal behaviour and mating system were first identified in the 1970s [24,142], and have since been discussed in several studies [24,141–

143,145]. Perhaps the two most influential papers considering the relationships between these traits are those by Greenwood [24] and Dobson [141]. In these papers, the authors detail how the mating strategies adopted by species are expected to influence dispersal pattern. Despite great interest and widespread acceptance of the hypothesised relationships, they remain largely untested, primarily due to the limitations described in Section 2.1.2 [142,145]. These hypotheses form the focus of the present study.

2.1.4.1. Polygyny, promiscuity and the evolution of male-biased dispersal

Greenwood [24] related MBD patterns in mammalian species to the adoption of mate defence strategies by males, but not females. When males adopt mate-defence reproductive strategies, they may maximise their reproductive success by maintaining primary access to females for a given time period [24]. This causes breeding access to females to become relatively limited, resulting in relatively high levels of local mate competition (LMC) between males [24].

In mammals, mate-defence strategies are commonly adopted by males in polygynous and promiscuous species [24,141,142,145]. In polygynous species, males adopt mate-defence strategies to gain primary access to a group of females (e.g. harem defence in red deer, *Cervus elaphus* [53]). By contrast, males in promiscuous species may adopt mate-defence strategies to attain sole access to females during the most fertile points of their reproductive cycle (as in the Western chimpanzee, *Pan troglodytes verus* [59]).

When males adopt mate defence strategies, and thus when LMC is high, some males will be excluded from accessing females. Juvenile and adolescent males are likely to be outcompeted by older, likely more dominant, males which are resident in their natal area [141]. Males which are excluded from breeding in their natal area can improve reproductive success by dispersing to access females in another area [24,141]. As such, high levels of LMC resulting from the adoption of mate-defence strategies are expected to drive the natal dispersal of males. By contrast, females are expected to remain philopatric. Where males adopt mate- defence strategies, the burden of parental care is typically on females, making their reproductive fitness more dependent on access to resources compared to males [24]. By remaining philopatric or dispersing short distances, females may take advantage of their advanced knowledge of resources in their natal area and could benefit from kin selection [24,141,145]. Dispersal is therefore predicted to be male-biased in species where males adopt mate-defence strategies.

Greenwood [24] used this logic to explain potential differences in dispersal distances between the sexes, but Dobson [141] later applied this same logic to explain differences in dispersal propensity. There is evidence for a relationship between polygyny and/or promiscuity and MBD in mammalian species in previous studies. For example, in an early comparative study, Dobson [141] found evidence of a link between MBD and polygynous and promiscuous mating systems in mammalian species. More recently, Brom *et al*. [196] produced models which suggested that the effect of polygyny and promiscuity on kin competition should cause MBD. As yet, no studies have investigated the association between polygyny and/or promiscuity and MBD using comparative methods with phylogenetic correction.

2.1.4.2. Monogamy and the evolution of either female-biased or equal dispersal

Monogamy (i.e. males and females mate at least primarily with a single partner) is relatively rare in mammals, but is common in birds [24,143]. Greenwood [24] associated monogamy in avian species with the adoption of resource-defence strategies by males. In such species, males attract mates by securing territories within or near their natal area which contain resources of sufficient quality and abundance to enable successful breeding. Thus, males are expected to remain philopatric or to disperse short distances [24,141]. Females are able to gain access to resources by dispersing and establishing exclusive access to a high quality territory, which is held by a male. Thus, FBD may be more likely to arise in monogamous species where males adopt resource defence strategies [24,141]. Males are more likely to adopt resource-defence strategies in avian species than in mammalian species. However, males of some mammalian species like the large treeshrew (*Tupaia tana*) [325] adopt resource-defence, rather than male-defence strategies.

Mabry *et al.* [145] recently used phylogenetic tests to determine whether FBD was more likely to arise in monogamous birds and mammals. The study used both measures of dispersal distance (as in Greenwood [24]) and dispersal propensity (as in Dobson [141]). The study found some evidence to suggest that FBD was more likely to evolve in monogamous mammalian species than in species with other mating systems. The authors acknowledged that the small sample size used ($n<60$ in all tests) may not reliably reflect the diversity of birds or mammals. Thus, further comparative tests using larger sample sizes are necessary to investigate this relationship.

Dobson [141] proposed an alternative hypothesis for an association between dispersal behaviour and monogamy in mammalian species. Dobson [141] suggested that in

monogamous mammalian species, both sexes are likely to experience similar levels of LMC and local resource competition (LRC). The levels of LMC will be comparable if the operational sex ratio (OSR) of the population (i.e. the relative number of receptive females and sexually active males [144]) is approximately 1:1, as neither sex will experience relatively limited access to mates. The levels of LRC should also be similar between the sexes because the reproductive investment of males and females at each breeding attempt will be focussed on one litter. Thus, members of each sex will require similar levels of access to resources associated with successfully rearing young [24,141]. This is likely to be particularly true where males exhibit paternal care, which is observed most frequently in mammals with a monogamous mating system [215,216]. As LRC and LMC are expected to be similar between the sexes in populations with a monogamous mating system, Dobson [141] predicted that the resultant dispersal pattern would not be sex-biased, thus monogamy in mammals may drive equal dispersal (ED) (i.e. dispersal propensity and/or distance will be similar between the sexes). Support for this hypothesis has been detected in previous comparative [141] and modelling [196] studies. However, as with hypotheses linked to MBD and promiscuity and/or polygyny, the hypothesis that monogamy is likely to drive ED has never been investigated using comparative methods with phylogenetic correction.

2.1.4.3. Paternal provisioning and equal dispersal

Maternal care is ubiquitous in mammals, primarily because gestation is internal and lactation is crucial in ensuring the survival of young in early stages, but paternal care is rare [146,147]. In species with no paternal care, competition for resources associated with rearing young will occur at least primarily between sexually mature females [24]. If females are excluded from these resources due to high levels of LRC, then they may be driven to disperse to gain access to these resources elsewhere [24]. If males do not provide paternal care, then their reproductive success will not be dependent on acquiring access to resources associated with parental care. Thus, males that do not provide parental care are not expected to be driven to disperse due to high levels of competition for resources specifically associated with parental care. However, if males do provide parental care, then LRC may be more similar between the sexes. LRC is most likely to be comparable between the sexes where males exhibit depreciable forms of care (i.e. forms of care in which investment in one litter/individual precludes investment in others [146,147]). One example of a depreciable form of care is provisioning behaviour. Provisioning behaviour may be indirect (i.e. males provide food to the mother during gestation) or direct (i.e. males provide food to offspring) [146]. Not all species that exhibit paternal provisioning behaviour are monogamous.

However, provisioning behaviour may be relatively more common in monogamous species than those with other mating systems because males are unlikely to be able to provide food to multiple independent litters. When males provision young, levels of LMC and LRC should thus be similar between the sexes. I therefore hypothesise that equal dispersal may be more likely to arise in species exhibiting paternal provisioning behaviour.

2.1.5. Aims of the present study

In this study, I use comparative approaches to investigate whether the natal dispersal pattern of a species is influenced by mating system. Specifically, I will examine whether promiscuity and/or polygyny drive the evolution of MBD, whether FBD and/or ED are more likely to arise in monogamous systems and if provisioning behaviour is likely to cause ED.

2.2. Methods

2.2.1. Data collection

Data on mating system and dispersal behaviour were collected from the literature. I only included data on either factor from reviews after checking the primary source(s) of the data. Data on provisioning behaviour in mammals were collected from reviews and primary sources by Professor Paula Stockley.

2.2.1.1. Data on dispersal pattern

The dispersal pattern of each species was classified according to the relative number of each sex dispersing from the natal group (i.e. the relative natal dispersal propensity of the sexes). Ideally, I would also have tested the hypotheses for dispersal distance in order to gain a full understanding of the biological significance of different dispersal patterns [165,176]. However, I found relatively few studies on mammalian species that explicitly considered dispersal distance, so I did not complete separate tests to investigate the effect of mating system on dispersal distance.

The level of data for both dispersal propensity and dispersal distance was limited in part by the difficulty in determining whether the dispersal pattern identified in studies referred to dispersal propensity (i.e. the number of animals dispersing) or distance. This was particularly true for genetic studies which inferred the dispersal pattern from data on population structure (e.g. [326]). Such studies considered whether related individuals of one sex were significantly closer than individuals of the other sex (i.e. whether there was more genetic structure in one sex). However, both dispersal propensity and distance may influence the

proximity of related individuals. Indeed, related animals of one sex may be relatively close either because they were more philopatric or because they dispersed shorter distances. I did not include data from studies unless it was clear that the dispersal pattern described a difference in dispersal propensity, rather than distance. Where possible, I attempted to corroborate a classification by collecting data obtained using multiple different methods.

The dispersal pattern of a species was categorised as male-biased (MBD), female-biased (FBD), equal dispersal (ED) or high philopatry (HP). A sex-biased dispersal pattern indicates that either males (MBD) or females (FBD) were significantly more likely to disperse from the natal group. If there was evidence that young usually dispersed from their natal site, but there was no evidence of a sex-bias in behaviour, I classified the species' dispersal pattern as ED. If natal dispersal was rare (<10% of individuals) or there was no evidence that it occurred (e.g. because mating occurred only during temporary movements), the dispersal pattern of the species was categorised as HP.

I only included data on natal dispersal to avoid confounding the results with data from breeding dispersal (i.e. permanent movements between sites in which animals breed). These two types of dispersal must be considered separately, as their causes and consequences differ [174–176]. Data on mammalian breeding dispersal behaviour was not considered in separate tests because data were scarce.

2.2.1.2. Data on mating system

Data on mating system were more readily available than data on dispersal pattern. I therefore collected data on mating system only where data on dispersal pattern were available for a species. Where possible, data on both mating system and dispersal pattern were taken from the same study. The mating system and dispersal pattern exhibited by a species may vary between populations of a species and between years within a population, dependent on factors including density [327–329]. Taking data on both factors from a single study maximised the likelihood that the data on each factor were related. If the dispersal pattern was linked to more than one mating system, then all possible mating systems were included in the dataset.

Species were classified as promiscuous if both males and females typically mated with multiple partners. If females typically mated with one male whilst males mated multiply, the species was considered to be polygynous. Species were considered polyandrous if females mated with multiple males, but males usually mated with a single female. Species that mated in male-female pairs were classified as monogamous if the majority of paternity was assigned

to the pair male. Where possible, data on the social mating system of a species was complemented with genetic data on mating system (as in Le Galliard *et al*. [193]).

Data on both mating system and dispersal pattern are available on a USB stick which is located in a pocket at the end of this thesis. Further information about the data is provided in Appendix 1.

2.2.1.3. Data on paternal provisioning

Data were collected using literature searches conducted in Web of Science. Males were considered to provision young if they provided food to mothers during offspring development and/or directly to young after birth. To be conservative, the absence of provisioning behaviour was only assumed where it was explicitly stated in studies. These data are available as supplementary material in Stockley and Hobson [146], which is provided at the end of the thesis.

2.2.1.4. Confidence in data classifications

I did not include data from species that exhibited a high degree of variation in dispersal behaviour and in mating system. This led to the exclusion of several species that had been included in previous dispersal studies. For example, several previous studies on dispersal behaviour have included the African wild dog (*Lycaon pictus*) and stated that it exhibits MBD [24,141,143], but McNutt [330] found evidence of FBD in a long term study. The extent of female dispersal is variable in *L. pictus* [331], so the dispersal pattern is likely to differ both between populations and between years. Where a high level of variation was evident, I attempted to match the dispersal pattern with a given mating system. If this was not possible, the species was not included in the dataset. This could result in a bias against relatively well studied species, for which variability is more likely to have been detected. The extent to which this could impact the results cannot be determined without further study of the dispersal behaviour and mating systems of mammalian species.

Although I was reasonably confident in the classifications for data on dispersal pattern and mating system for the species included in the dataset, the level of confidence of classifications varied between species. In recognition of this, I assigned a different level of confidence to different data. A low degree of confidence was assigned to data taken from preliminary studies with low sample sizes. Confidence was also low where a mating system and/or dispersal pattern was stated in at least one study, but the classification could not be corroborated. A high degree of confidence was assigned to data where there was direct

evidence of a given mating system and if the sample size in dispersal studies was likely to have been sufficient to have detected a bias. I considered the sample size to be sufficient if data was collected on at least 30 individuals, and/or if information in the paper suggested that there was enough data to have been able to detect a difference. I created two datasets which included data with different degrees of confidence. One dataset included all of the available data (hereafter the 'full' dataset) and one included only the data that was assigned a high degree of confidence (hereafter the 'conservative' dataset). In total, there were 218 species in the 'full' dataset and 168 species in the 'conservative' dataset. Information on the confidence assigned to data is available in the dataset provided on a USB stick which is located in a pocket on the back page of this thesis. Data on paternal provisioning behaviour was available for 71 species in the full dataset and 56 species in the conservative dataset.

2.2.2. Comparative methods

Selection pressures can cause the evolution of different traits to be correlated. Comparative methods are used to investigate these relationships [323]. Phylogenies, ideally with branch length data and full resolution, should be included in such analyses to account for the non-independence of traits resulting from the shared evolutionary history of species [324]. Here, I explain the methods used to test the hypotheses outlined in Section 2.1.4.

2.2.2.1. Formatting data and phylogenetic trees

During data collection, the species were classified as having one of four dispersal patterns (MBD, FBD, ED or HP) and at least one of four mating systems (promiscuity, polygyny, polyandry or monogamy) (see Section 2.2.1.). Each hypothesis considers an association between a dispersal pattern and at least one mating system. To test the hypotheses, I reclassified the data as binomial variables to reflect the presence or absence of a given dispersal pattern or mating system(s). For example, to test whether MBD was linked to polygyny, I classified the dispersal pattern data as MBD or not (i.e. MBD was either present or absent), and classified mating system as polygynous or not. If multiple mating systems were associated with a given dispersal pattern, all classifications were included in tests. For instance, if there was evidence that a species exhibited both polygyny or monogamy, then in tests considering polygyny, polygyny was classified as both absent and present in that species. This allowed the mating system trait to adopt either state during subsequent analyses.

The APE package [332] within R v. 3.1.0 [333] was used to prune phylogenetic trees to include only the species within each dataset (i.e. the 'conservative' and 'full' datasets). The IUCN Red

List dataset v. 2015-4 [334] was used to match species in both of those datasets with the species in the tree(s) used in each test.

2.2.2.2. Maximum likelihood methods

I used maximum likelihood (ML) functions within the Discrete module of BayesTraits V. 2.0 [335] to conduct preliminary tests of the hypotheses. Maximum likelihood tests were used to determine whether a dispersal pattern (e.g. MBD) was associated with a given mating system (or mating systems) (e.g. polygyny, or both polygyny and promiscuity) as expected. Specifically, the tests examined whether each pair of traits were likely to have evolved independently (i.e. the traits followed an independent model of evolution), or whether their evolution was correlated (i.e. the traits followed a dependent model of evolution). In maximum likelihood tests, the log-likelihood (Lh) value of a model assuming dependent evolution of two traits is compared to the Lh of a model assuming independent evolution. This generates a maximum likelihood (ML) value. The maximum likelihood value was calculated as follows (taken from Pagel and Meade [335]):

ML value = 2*(Lh dependent model- Lh independent model)

I used chi-squared tests to determine whether the ML value represented a significant difference between the likelihood values of the two models. The number of degrees of freedom is equal to the difference in the number of transition rates in the models being compared. Dependent models have eight transition rates, whilst independent models have four [335], thus there were four degrees of freedom in all ML tests.

Tests were conducted using both the 'all' and 'conservative' datasets. The best estimate mammalian supertree from Fritz *et al*. [336] was used in all tests. All polytomies in the tree were randomly resolved using the APE package [332] within R v. 3.1.0 [333] before tests.

2.2.2.3. MCMC methods

I used a Markov-chain Monte Carlo (MCMC) sampling algorithm with a reversible jump (RJ) procedure to further test the hypotheses [337,338]. These tests were conducted using the MCMC functions in the Discrete module of BayesTraits V. 2.0 [335]. Whilst ML tests had been conducted using a single randomly resolved tree, all MCMC tests were conducted using a sample of 100 trees. These trees were taken from Kuhn *et al*. [339], the polytomies in these trees had been resolved using life-death models. An exponential prior distribution was used as previous tests suggested that transition rates were low [340]. The prior was seeded from a range of 0-2. Rate deviation values for each model were approximated using Autotune

within BayesTraits V. 2.0 [335]. Each MCMC chain was run for 5 million iterations with sampling occurring every 5000 iterations. To ensure that the model had reached convergence, the first 100,000 iterations were excluded as the burn-in period. Convergence was assessed visually using Tracer v. 1.6. [341].

Log BayesFactor tests were used to determine whether traits evolved independently or dependently. In these tests, the harmonic mean value of an MCMC chain assuming a pair of traits evolved independently was compared to that of a chain assuming dependent evolution. The resultant log BayesFactor score was interpreted according to the information provided in Table 2.1 (taken from Pagel and Meade [335]). To be conservative, the evolution of two traits was only assumed to be correlated where the log BayesFactor score suggested that there was 'strong evidence' for dependent evolution. This method was taken from Pagel and Meade [335].

Table 2.1. Interpretation of log BayesFactor scores. Table modified from that in Pagel and Meade [335]. Different log BayesFactor scores constitute a certain degree of evidence for dependent evolution. Interpretation represents the degree of evidence for dependent evolution, which may be inferred from different Log BayesFactor scores.

Log BayesFactor score	Interpretation
$x < 2$	Weak evidence
$2 \leq x < 5$	Positive evidence
$5 \leq x < 10$	Strong evidence
$x \geq 10$	Very strong evidence

The results of log BayesFactor tests can show whether there is a relationship between traits (i.e. whether the evolution of traits is dependent or independent). However, log BayesFactor tests do not show how related traits (i.e. those for which there is evidence of correlated evolution) are likely to coevolve. I employed the method outlined in Opie *et al*. [340] to investigate how related traits were associated.

If there was evidence of dependent evolution in log BayesFactor tests, the MCMC chain assuming dependent evolution was run a total of five times. The chain with the median harmonic mean value was used to determine average transition rates, Z-scores, transition rate combinations and ancestral state values. These values were calculated using the post-convergence portion of the run. The likelihood of a transition occurring can be inferred from the Z-score, with higher Z-scores indicating that a transition is less likely to occur. Transition

rate values represent the relative frequency of a transition. Transition rate combinations indicate which transitions are likely to occur at the same rate and which transitions are not predicted to occur (see Appendix 1 for further information and transition rate combination results). Ancestral state values represent the probability that a particular combination of traits is the ancestral state. In dependent models there are four possible trait combinations: (i) trait A present and trait B absent, (ii) trait A present and trait B present, (iii) trait A absent and trait B absent, and (iv) trait A absent and trait B present.

2.3. Results

2.3.1. Is male-biased dispersal likely to arise in polygynous and/or promiscuous species?

In this section I present the results of tests completed to investigate possible associations between MBD and mating system in mammals. Specifically, I examine whether (1) polygyny drives the evolution of MBD, (2) promiscuity drives the evolution of MBD and (3) MBD is likely to evolve in species in which males are likely adopt mate-defence strategies (i.e. polygynyous or promiscuous species). These hypotheses are reflective of the predictions and subsequent tests completed in Greenwood [24] and Dobson [141].

2.3.1.1. Maximum likelihood tests

The results of maximum likelihood (ML) tests of hypotheses regarding potential relationships between MBD and mating system in mammalian species are provided in Table 2.2. I found no evidence of dependent (correlated) evolution between MBD and either promiscuity or polygyny when the mating systems were considered separately. This suggests that MBD evolves independently of either mating system. However, there was evidence of dependent evolution when polygyny and promiscuity were combined (such that all mating systems in which males are likely to adopt mate-defence strategies were considered together). This result suggests that the evolution of MBD may be associated with the adoption of mate-defence strategies by males. The results did not vary according to the dataset used to generate models, and thus the level of confidence assigned to data classifications.

Table 2.2. Results of maximum likelihood tests investigating whether the evolution of male-biased dispersal is associated with the evolution of polygyny and/or promiscuity in mammalian species. Male-biased dispersal was the dispersal pattern considered in each test. The evolution of male-biased dispersal was compared to that of mating system, specifically polygyny (Po), promiscuity (Pr) or both polygyny and promiscuity (Pr and Po). Tests were completed using either the 'all' or 'conservative' dataset. The log-likelihood (Lh) values of models assuming dependent or independent evolution are referred to as Dep Lh and Indep Lh, respectively. The ML value refers to the maximum likelihood value generated in tests. The model of evolution indicates whether the traits were likely to evolve independently (indep) or dependently (dep). Values are given to three decimal places.

Mating system	Dataset used	Dep Lh	Indep Lh	ML value	Df	P	Model of evolution
Po	All	-256.840	-259.148	4.616	4	0.329	Indep
Po	Conservative	-199.311	-202.388	6.154	4	0.188	Indep
Pr	All	-254.681	-255.128	0.893	4	0.925	Indep
Pr	Conservative	-196.681	-198.928	4.495	4	0.343	Indep
Pr and Po	All	-191.653	-198.384	13.463	4	0.009	Dep
Pr and Po	Conservative	-146.085	-154.661	17.152	4	0.002	Dep

2.3.1.2. Log BayesFactor tests

The ML tests provided preliminary evidence about the hypothesised relationships between MBD and polygyny and/or promiscuity. I completed log BayesFactor (BF) tests to further investigate these hypotheses. As in the ML tests above (Table 2.2), I found no evidence of correlated evolution between male-biased dispersal (MBD) and polygyny (Table 2.3). There was 'strong' evidence of correlated evolution between MBD and promiscuity, but only when all the available data were included in tests (Table 2.3). To be conservative, I only considered the evolution of two traits to be correlated where there was 'very strong' evidence for dependent evolution (see Table 2.1 for information regarding the interpretation of BF values). I therefore concluded that MBD and promiscuity evolved independently. There was 'very strong' evidence of dependent evolution between MBD and mating system when polygyny and promiscuity were considered together, but only when the conservative dataset was used (Table 2.3). This latter result was thus partly consistent with initial expectations and with the results of ML tests (Table 2.2).

Table 2.3. Results of log BayesFactor tests investigating whether the evolution of male-biased dispersal is associated with the evolution of polygyny and/or promiscuity in mammalian species. Male-biased dispersal was the dispersal pattern considered in each test. The evolution of male-biased dispersal was compared to that of mating system, specifically polygyny (Po), promiscuity (Pr) or both polygyny and promiscuity (Pr and Po). Tests were completed using either the 'all' or 'conservative' dataset. The harmonic mean (HM) values of models assuming dependent and independent evolution of traits are shown in dep HM and indep HM, respectively. BF represents the log BayesFactor value generated in each test. The level of evidence for dependent evolution was inferred using the information in Table 2.1.

Mating system	Dataset used	Dep HM	Indep HM	BF	Evidence for dependent evolution
Po	All	-262.845	-262.089	-1.513	Weak
Po	Conservative	-204.128	-203.541	-1.174	Weak
Pr	All	-257.564	-261.166	7.205	Strong
Pr	Conservative	-203.568	-204.337	1.538	Weak
Pr and Po	All	-200.829	-202.018	2.378	Positive
Pr and Po	Conservative	-152.679	-158.611	11.864	Very strong

2.3.1.3. Determining the nature of the association

I used information from models to further investigate the nature of the relationship between MBD and mating system (promiscuity and polygyny), which was evident in BF tests (see Table 2.3). I expected that the presence of polygyny and/or promiscuity would drive the evolution of MBD. The results in Figure 2.1 show that the most likely ancestral state was MBD with polygyny or promiscuity. The most likely transition from the ancestral state was a loss of MBD. This suggests that, inconsistent with the initial expectations, changes in dispersal pattern precede changes in mating system.

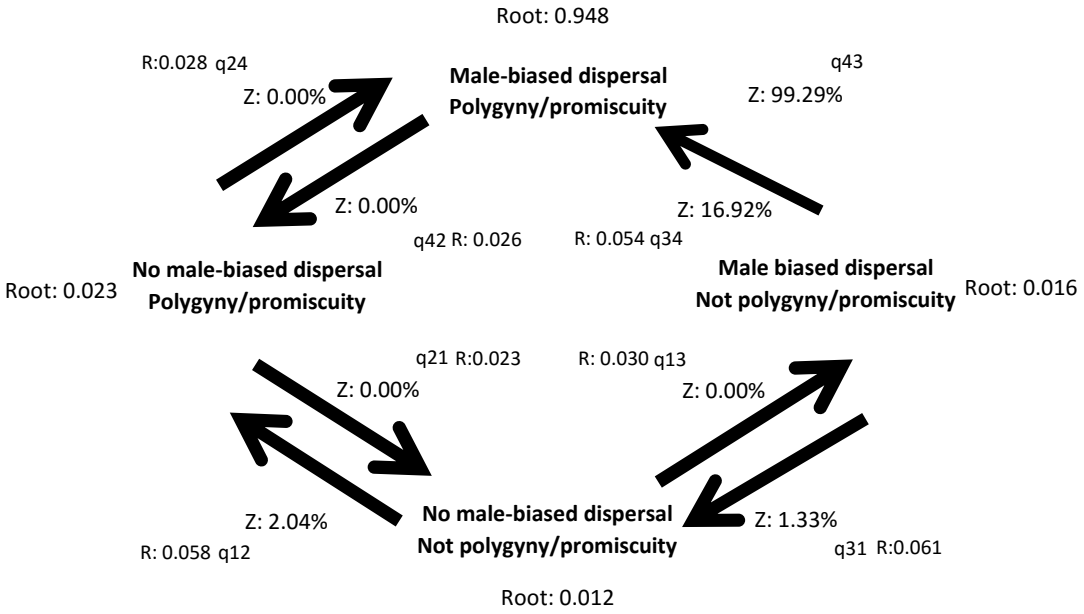

Figure 2.1. Coevolution between male-biased dispersal and a polygynous or promiscuous mating system in mammalian species. Ancestral state values are shown as root values, which indicate the proportion of the post-convergence portion of the model for different states. Transition rate names are denoted as q(xy). Z-values reflect the percentage of visits in the post-convergence portion of the run assigned to zero. Arrows represent transitions between states, and are scaled to represent the probability of a transition (determined using the Z-values). Arrows are not included if Z-values are over 90%. R values represent mean transition rates, and are only given where the Z value associated with a transition is less than 25%.

2.3.2. Is female biased dispersal more likely to arise in monogamous mammals?

In this section I will investigate the hypothesis that monogamy drives the evolution of female-biased dispersal (FBD).

2.3.2.1. Maximum likelihood tests

I found no evidence that the evolution of female-biased dispersal (FBD) was associated with that of monogamy using ML tests. The result did not vary according to the dataset used in each test (Table 2.4).

Table 2.4. Results of maximum likelihood tests investigating whether the evolution of female-biased dispersal is associated with the evolution of monogamy in mammalian species. Tests were completed using either the 'all' or 'conservative' dataset. The log-likelihood (Lh) values of models assuming dependent or independent evolution are referred to as Dep Lh and Indep Lh, respectively. The ML value refers to the maximum likelihood value generated in tests. The model of evolution indicates whether the traits were likely to evolve independently (indep) or dependently (dep). Values are given to three decimal places.

Dataset used	Dep Lh	Indep Lh	ML value	Df	P	Model of evolution
All	-124.768	-126.505	3.473	4	0.482	Indep
Conservative	-101.695	-103.361	3.331	4	0.504	Indep

2.3.2.2. Log BayesFactor tests

Consistent with the results of ML tests (Table 2.4), I found no evidence of correlated evolution between FBD and monogamy in BF tests (Table 2.5). As there was no evidence of an association between FBD and monogamy, I did not investigate the relationship between these traits further.

Table 2.5. Results of log BayesFactor tests investigating whether the evolution of female-biased dispersal is correlated with the evolution of monogamy in mammalian species. Tests were completed using either the 'all' or 'conservative' dataset. The harmonic mean (HM) values of models assuming dependent and independent evolution of traits are shown in dep HM and indep HM, respectively. BF represents the log BayesFactor value generated in each test. The level of evidence for dependent evolution was inferred using the information in Table 2.1.

Dataset used	Dep HM	Indep HM	BF	Evidence for dependent evolution
All	-136.236	-131.675	-9.123	Weak
Conservative	-108.226	-109.917	3.382	Positive

2.3.3. Is equal dispersal more likely to arise in monogamous mammals?

Here I investigate the hypothesised association between monogamy and equal dispersal (ED) in mammalian species.

2.3.3.1. Maximum likelihood tests

There was evidence of correlated evolution between monogamy and equal dispersal in maximum likelihood tests (Table 2.6). The result was not dependent on the dataset used to generate models.

Table 2.6. Results of maximum likelihood tests investigating whether the evolution of equal dispersal is associated with the evolution of monogamy in mammalian species. Tests were completed using either the 'all' or 'conservative' dataset. The log-likelihood (Lh) values of models assuming dependent or independent evolution are referred to as Dep Lh and Indep Lh, respectively. The ML value refers to the maximum likelihood value generated in tests. The model of evolution indicates whether the traits were likely to evolve independently (indep) or dependently (dep). Values are given to three decimal places.

Dataset used	Dep Lh	Indep Lh	ML value	Df	P	Model of evolution
All	-169.455	-175.952	12.993	4	0.011	Dep
Conservative	-131.431	-139.653	16.446	4	0.002	Dep

2.3.3.2. Log BayesFactor tests

Consistent with the results of ML tests (Table 2.6), there was evidence for dependent evolution between ED and monogamy in BF tests (Table 2.7).

Table 2.7. Results of log BayesFactor tests investigating whether the evolution of equal dispersal is correlated with the evolution of monogamy in mammalian species. Tests were completed using either the 'all' or 'conservative' dataset. The harmonic mean (HM) values of models assuming dependent and independent evolution of traits are shown in dep HM and indep HM, respectively. BF represents the log BayesFactor value generated in each test. The level of evidence for dependent evolution was inferred using the information in Table 2.1.

Dataset used	Dep HM	Indep HM	BF	Evidence for dependent evolution
All	-175.902	-185.032	18.260	Very strong
Conservative	-139.049	-149.889	21.680	Very strong

2.3.3.3. Determining the nature of the association

I used information from models assuming dependent evolution to further investigate the nature of the relationship between ED and monogamy in mammalian species. The data provided in Figure 2.2 indicates that the most likely ancestral state was a dispersal pattern besides ED and a mating system besides monogamy (i.e. 'not equal dispersal' and 'not

monogamy'). This is consistent with previous findings related to the relationship of MBD and polygyny/promiscuity (Figure 2.1). The data also suggests that evolutionary transitions in dispersal pattern were likely to precede those in mating system. Specifically, equal dispersal was more likely than monogamy to be gained from the ancestral state. The results generated using the 'full' dataset are consistent with those generated using the 'conservative' dataset (evident from a comparison of Figures 1.2A and 1.2B).

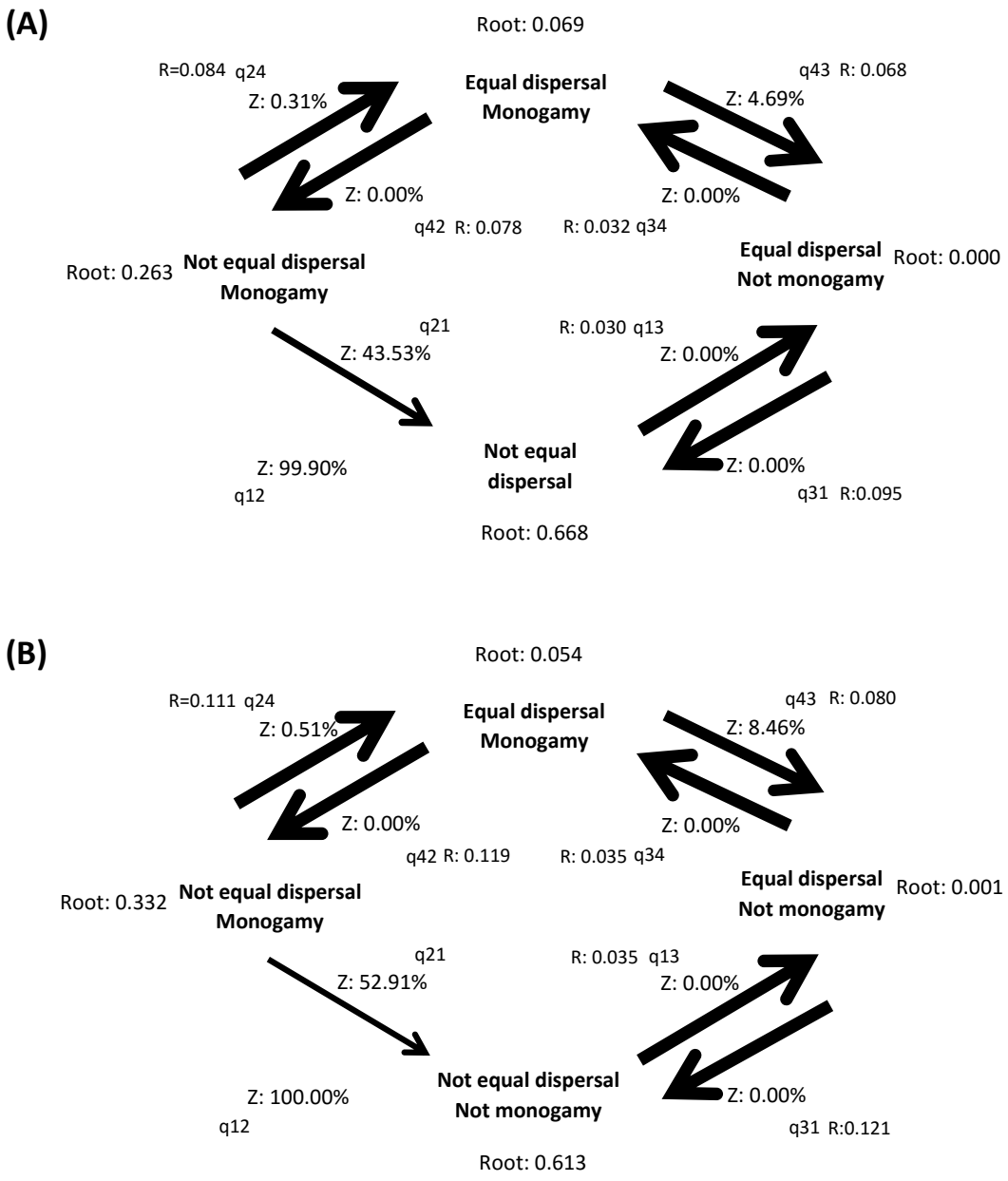

Figure 2.2. Coevolution between equal dispersal and monogamy in mammalian species (A) model generated using the 'full' dataset (B) model generated using the 'conservative' dataset. See main text for details on the datasets used. Ancestral state values are shown as root values, which indicate the proportion of the post-convergence portion of the model for different states. Transition rate names are denoted as q(xy). Z-values reflect the percentage of visits in the post-convergence portion of the run assigned to zero. Arrows represent transitions between states, and are scaled to represent the probability of a transition (determined using the Z-values). Transitions with Z-scores over 50% are represented by dashed lines and no lines are shown if Z values are over 90%. R values are mean transition rates, and are only given where the Z values associated with a transition is less than 25%.

2.3.4. Is equal dispersal more likely to arise in species with paternal provisioning?

Finally, I tested the hypothesis that the presence of paternal provisioning behaviour was likely to drive ED in mammalian species.

2.3.4.1. Maximum likelihood tests

There was no evidence that the evolution of equal dispersal (ED) was linked to that of paternal provisioning behaviour in ML tests (Table 2.8). The result did not vary according to the dataset used to generate models.

Table 2.8. Results of maximum likelihood tests investigating whether the evolution of equal dispersal is associated with the evolution of paternal provisioning behaviour in mammalian species. Tests were completed using either the 'all' or 'conservative' dataset. The log-likelihood (Lh) values of models assuming dependent or independent evolution are referred to as Dep Lh and Indep Lh, respectively. The ML value refers to the maximum likelihood value generated in tests. The model of evolution indicates whether the traits were likely to evolve independently (indep) or dependently (dep). Values are given to three decimal places.

Dataset used	Dep Lh	Indep Lh	ML value	Df	P	Model of evolution
All	-63.857	-66.945	6.176	4	0.186	Indep
Conservative	-52.879	-54.857	3.956	4	0.412	Indep

2.3.4.2. Log BayesFactor tests

There was 'positive' evidence for dependent evolution between paternal provisioning behaviour and monogamy in mammalian species (Table 2.9). However, given the conservative approach employed in this study, this was considered insufficient evidence for a relationship between these traits. This result is consistent with the results of ML tests on these traits (Table 2.8). As there was insufficient evidence for a relationship, no further investigations were completed for this hypothesis.

Table 2.9. Results of log BayesFactor tests investigating whether the evolution of equal dispersal is correlated with the evolution of paternal provisioning behaviour in mammalian species. Tests were completed using either the 'all' or 'conservative' dataset. The harmonic mean values of models assuming dependent and independent evolution of traits are shown in dep HM and indep HM, respectively. BF represents the log BayesFactor value generated in each test. The level of evidence for dependent evolution was inferred using the information in Table 2.1.

Dataset used	Dep HM	Indep HM	BF	Evidence for dependent evolution
All	-72.568	-74.778	4.420	Positive
Conservative	-61.815	-64.240	4.850	Positive

2.4. Discussion

The aim of this chapter was to investigate hypothesised associations between natal dispersal patterns and mating systems in mammalian species. I specifically considered the relative numbers of each sex dispersing from the natal area (i.e. natal dispersal propensity). Here, I discuss the implications of the results of this study, before making general conclusions and suggesting possible future directions for studies in this area.

2.4.1. Male-biased dispersal and polygyny and/or promiscuity

Males of polygynous and promiscuous mammalian species typically adopt mate-defence strategies [24,141]. Where such strategies are adopted, individual males restrict access to females, resulting in high levels of LMC between males which may lead to high levels of male dispersal. However, females are not expected to be subject to high levels of LMC, and may benefit from philopatry as they will have greater knowledge of the resources in their natal area. As such, the adoption of mate-defence strategies in mammals is thought to drive MBD, with males dispersing further and/or more frequently from the natal area than females [24,141].

Contrary to expectations, I found no evidence that the evolution of either polygyny or promiscuity causes males to be more likely than females to disperse from the natal area/group. However, there was some evidence of dependent evolution between MBD and mating system when polygyny and promiscuity were considered together, but only when using the 'conservative' dataset to generate models (Tables 2.2. and 2.3). This last result is somewhat consistent with the results of Dobson [141].

I initially expected much stronger evidence for a relationship between polygyny and/or promiscuity in MBD, given the results of Dobson [141] and the high coincidence of these traits (i.e. MBD and either promiscuity or polygyny) in mammalian species, both of which have contributed to the wide-spread acceptance of the proposed relationship. However, the observations of Greenwood [24] may provide an explanation for the relatively low level of evidence for an association between MBD and polygyny and/or promiscuity. Specifically, Greenwood [24] noted that there was a high degree of variation in the mating strategies adopted by mammals that are classified as having the same broad mating system (e.g. polygyny). Indeed, neither polygyny nor promiscuity is inextricably linked with the adoption of mate-defence strategies by males [12,144,342]. For example, in the greater white-lined bat (*Saccopteryx bilineata*) males adopt resource-defence strategies, and harems of females form in feeding territories that are defended by a single male. The females within the harem primarily mate with the male that is resident in the feeding territory, thus making the species polygynous [342,343]. Thus, whilst the adoption of mate-defence strategies by males is likely to lead to high levels of LMC between males and therefore MBD, mating system itself may be a poor predictor of MBD in mammals [24].

It is possible that the only link detected between MBD and mating system in this study (i.e. MBD and polygyny and promiscuity) resulted due to the high incidence of polygyny, promiscuity and MBD in mammalian species. The high incidence of these traits in mammals could have caused an association to appear evident although the traits were not associated. Alternatively, the association may have resulted because the adoption of mate-defence strategies by males was common in species with polygynous or promiscuous mating systems and MBD. This would not necessarily be surprising given the high incidence of the adoption of mate-defence strategies by mammalian males, particularly in polygynous species [12,144]. However, if this were the case, I would have expected evidence of a relationship between polygyny and MBD, making this latter explanation unlikely.

The results presented here investigating the relationship between MBD and mating system are inconsistent with those from the early comparative study by Dobson [141]. The results of that earlier study were based on a relatively small dataset, which did not account for the evolutionary relatedness of species. Thus, the results of the present study are likely to be more reliable than those in Dobson [141], as I accounted for non-independence of species in analyses and used a larger dataset, which is more likely to be representative of mammals [145]. This conclusion is supported by the fact that the results presented here are consistent with the results of more recent studies. For example, Le Galliard *et al*. [193] found no

association between mating system and MBD in arvicoline rodents. Moreover, information in computer simulations by Brom *et al*. [196] suggest that polygynous and promiscuous species are only likely to exhibit MBD where the costs of dispersal are low. The cost of dispersal is expected to be greater for males that adopt resource-defence rather than mate-defence strategies, because males adopting resource-defence strategies are more likely to benefit from remaining philopatric.

Taken together, the results of recent studies, including those presented here, indicate that future studies should attempt to directly associate MBD and the adoption of mate-defence strategies by males, rather than mating system *per se*.

2.4.2. Monogamy and the evolution of female-biased or equal-dispersal

2.4.2.1. Monogamy and female-biased dispersal

Greenwood [24] noted that avian species were often monogamous and that males frequently adopted resource defence-strategies to attract mates. Males that adopt resource defence strategies may benefit from increased familiarity with resources, and may thus remain philopatric. Females are expected to disperse in such species to locate a mate and to secure primary access to the associated breeding territory [24]. Thus, Greenwood [24] expected that the adoption of resource-defence strategies would be associated with monogamy and with FBD, at least in avian species. Mabry *et al*. [145] recently found evidence that FBD was more likely to arise in monogamous mammalian lineages, indicating that monogamy may cause FBD in mammalian species.

Contrary to expectations based on the results by Mabry *et al*. [145], I found no evidence of an association between FBD and monogamy in mammalian species (Tables 2.4 and 2.5). The results in Mabry *et al*. [145] were described as 'preliminary' by the authors who acknowledged that the sample size (n<60) may poorly reflect the diversity of mammalian species. The sample sizes used in the present study are much larger ('full' dataset: n=218, 'conservative' dataset: n=168), so the results presented are more likely to be reliable. Although the results are inconsistent with initial expectation, they are not necessarily surprising. Indeed, in stark contrast to avian species [24,141,142,145], males in monogamous mammalian species rarely adopt resource-defence strategies [325]. Thus, if FBD results because males adopt resource-defence strategies, as suggested by Greenwood [24] for birds, then it should only be evident in very few species, which may be insufficient to enable the detection of an association.

2.4.2.2. Monogamy and equal dispersal

Dobson [141] hypothesised that the levels of LMC and LRC experienced by members of each sex, and thus the propensity for dispersal, would be similar in monogamous mammalian species. If this was the case, then monogamy should cause ED. Consistent with this hypothesis, I found evidence of coevolution between monogamy and ED in both maximum likelihood and Log BayesFactor tests. However, contrary to expectation, the results indicated that changes in mating system preceded evolutionary transitions in dispersal pattern (Figure 2.2.), indicating that monogamy does not cause ED.

Although the levels of LRC and LMC were not estimated in this study, it is likely that the levels experienced by each sex were similar in monogamous species, as members of both sexes are likely to invest primarily in one litter [141]. Thus, it is unsurprising that monogamy and ED coevolve. However, there is little reason to expect ED should cause monogamy, as suggested in the results presented in Figure 2.2. This may be an indication that the traits only covary, and that the relationship results because both traits are influenced by a common factor such as the distribution of resources, including mates. Further investigation would be required to determine whether this is likely to be the case.

The finding that ED and monogamy coevolve is consistent with the results of previous studies by Dobson [141] and Brom *et al*. [196]. However, to my knowledge, no other study has investigated the directionality of the association between ED and monogamy. Ideally, future studies will also consider the directionality of this relationship in mammals, and in other species, to assess the reliability of the conclusions drawn herein.

2.4.3. Paternal provisioning behaviour and equal dispersal

Where males provide depreciable forms of care, such as provisioning behaviour, they are more likely to invest primarily in one litter [146]. Thus, males that provision young either directly or indirectly, are expected to experience levels of LRC and LMC comparable to those of females in mammalian species [24,141,146,147]. ED was therefore expected to be more likely to evolve where males provision young. However, I found no evidence of dependent evolution between paternal provisioning behaviour and ED (Tables 2.8 and 2.9).

The lack of a relationship may result because the levels of LRC and LMC differ between the sexes. Although paternal provisioning behaviour is regularly associated with monogamy, it may also occur in other mating systems. Thus, the levels of LMC are not necessarily similar in each sex. Moreover, the level of LRC may differ between sexes if the males do not invest as

much as females in litters. This may occur if, for example, males provision only occasionally, in which case males may be less affected by LRC than females. It is also possible that the relatively small sample sizes used in this study (full dataset n= 71, conservative dataset n= 56) precluded the detection of an association. The small sample sizes resulted because there were relatively few mammalian species for which data on dispersal pattern, mating system and paternal provisioning behaviour were available. Future studies with greater levels of data may be able detect an association.

Potential relationships between dispersal behaviour and parental care in general remain understudied. There are two recent studies, both by Kuijper and Johnstone [344,345], which consider the relationship between parental care and dispersal. The more recent of these studies [345] indicates that the level of parental care may vary according to sex-biases in dispersal. Specifically, models generated using computer simulations indicate that the more philopatric sex is likely to provide significantly more parental care than the more dispersive sex [345]. This would be consistent with the pattern generally observed in mammals, as females are typically the more philopatric sex and are more likely to provide parental care [24,141–143,145,147,207,214]. However, the results of that study should be considered with care, as the publication is yet to be subject to peer review.

2.5. Conclusions

The dispersal patterns of mammalian species have frequently been associated with mating system. The hypothesised relationships between mating system and dispersal pattern in mammalian species have been central in dispersal theory over the last four decades. One of the most widely accepted associations is that between polygyny and male-biased natal dispersal. However, I found little evidence for an association between male-biased dispersal and polygyny and/or promiscuity. This is possibly because male-biased dispersal is more likely to arise due to the adoption of mate-defence strategies by males, rather than polygyny or promiscuity in particular. I also investigated potential relationships between monogamy and dispersal. Another key hypothesis in mammals is that monogamy will lead to equal dispersal, with males and females equally likely to disperse from the natal area. I found some evidence in support of this hypothesis, but not for the alternative hypothesis for a relationship between female-biased dispersal and monogamy. Although there was a relationship between equal dispersal and monogamy, my results suggest that the relationship may represent covariation, rather than causation. Surprisingly, there was no evidence for an association between equal dispersal and paternal provisioning behaviour. This may be due

to the relatively small sample size used in this study. Taken together, the results presented in this study suggest that mating system is a poor predictor of dispersal behaviour. Future studies should instead attempt to explain mammalian dispersal behaviour in relation to the adoption of different reproductive strategies (e.g. resource- or mate-defence strategies) by males and females, rather than mating system.

Chapter 3: Investment in pre- and post- copulatory competition is related to male dispersal behaviour in promiscuous mammals.

Abstract

Males of mammalian species typically compete for mates, rather than other resources related to reproduction. Mate competition between males may occur pre- and/or post-copulation. Investment in either strategy can be influenced by the relatedness of competing males, with investment relatively lower where males compete among more kin. I used phylogenetic analyses to investigate how the relatedness of competing males influences investment in precopulatory and postcopulatory competition in mammalian species. Data on male dispersal behaviour were used to approximate the relatedness of competitors. Contrary to theoretical predictions, I found that the mass of testes relative to body mass, and thus ejaculate investment, was seemingly increased where males are likely to compete with kin. This may be because the risk of sperm competition is greater where the relatedness of competing males is high. There was evidence of an association between investment in precopulatory competition and male relatedness, but only when levels of investment were relatively high. The results indicate that future studies should incorporate the risk of sperm competition and consider other measures of pre-copulatory competition.

3.1. Introduction

3.1.1. Sexual selection, sexual dimorphism and sex roles

Darwin [2] introduced the concept of sexual selection as an explanation for sexual dimorphism within a species. A species can be considered sexually dimorphic if one sex is larger or possesses traits that are absent in the other [44,346,347]. Sexual dimorphism is expected to arise where a trait and/or larger size confers an advantage in reproductive competition for individuals of one sex [347]. The armaments of ungulates (i.e. horns and antlers) are a commonly used example of a sexually selected trait that confers an advantage. In many ungulate species only males possess armaments, and these armaments confer an advantage in male-male contests [43,44,53].

Males and females each attempt to maximise reproductive success, but the sexes do not invest equally in reproduction [7,348–351]. Where each sex has a different 'role' in reproduction (sex-role), there will be inter-sexual variation in reproductive strategy. This causes sexual selection to act differently in each sex, and results in sexual dimorphism [7,348–351]. For example, in many species, females invest more in parental care than males

[146,207]. In such species, females maximise reproductive success by ensuring offspring survival, whilst males maximise reproductive success by mating with as many females as possible. Females are likely to be receptive to males when they are not providing maternal care to a litter, whilst males will be receptive throughout the breeding period. The difference in receptivity between the sexes means that the operational sex ratio (OSR) (i.e. the ratio of receptive females to sexually mature males) will be skewed towards males [144]. As females will be the more 'limited' sex (i.e. there will be fewer receptive females than males), competition for mates will be primarily between males [144]. As males compete heavily for mates, selection will favour attributes that confer an advantage in male-male competition, which could include armaments and/or increased body size [144].

3.1.2. Mate competition in males

Mate competition in males may occur either before or after copulation with females (otherwise known as pre- and post-copulatory competition, respectively) [52,106,109,124,126]. Relative investment in different strategies will be evident in traits that are likely to confer an advantage with a given strategy. For example, weaponry confers an advantage in overt male-male contests, so males that participate in such contests may have some form of armament [43,53]. The level of investment in these traits will largely depend on the level of mate competition experienced by individuals (i.e. the amount of competition for access to mates or to maximise paternity within litters) [74,79,84,90,95,109,151]. This can be influenced by factors such as mating system, which is closely related to reproductive competition [12,24,84,151]. Investment in mate competition may also be impacted by the relatedness of competitors [117,352,353]. The relatedness of competitors is determined by dispersal behaviour, as males are likely to compete among kin unless related males disperse separately from a natal area [142,196].

3.1.3. Traits associated with investment in pre- and post-copulatory competition

Here I outline some of the causes and consequences of investment in pre- and post-copulatory competition. I focus particularly on mammalian species, which will be the focus of this study.

3.1.3.1. Pre-copulatory competition

In pre-copulatory contests, males compete to secure access to a female, or group of females [109]. Males may compete by displaying to females using ornaments; secondary sexual characters that have developed to convey information regarding the quality of a male. Access

to mates in these species is dictated by female choice (i.e. mate choice/intersexual selection) [354]. In other species, pre-copulatory competition comprises primarily of overt, aggressive interactions between males. Males may develop armaments which maximise their effectiveness in such conflicts. Examples of armaments include antlers (as in red deer, *Cervus elaphus* [43]), horns (e.g. horned beetles in the genus *Onthophagus* [127]) and elongated/strengthened limbs (as in the Australian quacking frog, *Crinia georgiana* [124]). Larger body size can also confer an advantage in inter-male combat. This may lead to sexual size dimorphism (SSD), with males becoming larger than females (i.e. male-biased SSD) [95,126]. Indeed, in a variety of species including mammals [346], birds [151] and snakes [355] the level of inter-male conflict is closely associated with the level of SSD.

The outcome of inter-male contests influences which males gain access to females. Typically, males that are victorious in contests secure primary access to at least one female, but males must continually outcompete others to maintain this access. Males will lose access if they lose in a subsequent contest. Victorious males will attempt to exclude all other males from mating with the female(s) with which they have mated. The ability of males to exclude competitors will depend on several factors, including female dispersion and female group size [12]. If female dispersion is high, then males may compete to secure access to one female [12]. Males that secure access to one female often guard their mate to ensure that they sire all the offspring produced by that female [95]. Males that secure access to several females are less able to prevent extra-group copulation, as males are less able to guard multiple mates effectively [92].

3.1.3.2. Post-copulatory competition

If multiple males mate with the same female, their sperm will compete to fertilise an ovum. This is termed sperm competition [52,106,109,356,357]. Males can attempt to maximise their share of paternity under sperm competition by investing more in sperm production [117]. That is, if the probability of fertilising an ovum is dictated by a fair raffle process (i.e. each sperm has an equal chance of fertilising the ovum), males may increase their chances of paternity by investing more sperm in their ejaculates [106,117,120,356]. Sperm are produced in the testes, and testes size increases both with body size and with sperm production levels [67,80,84,106,153]. Males that produce more sperm, and thus allocate more sperm in ejaculates, often have larger testes relative to their body size, so investment in post-copulatory competition may be inferred from relative testes mass (evidence for mammals reviewed in [65]).

3.1.4. Factors affecting investment in mate competition between males

3.1.4.1. Mating system

The mating system of a species is closely related to the levels of reproductive competition experienced by members of each sex. The mating systems of mammalian species are ultimately determined by the mating behaviour adopted by males and females [12]. The mating behaviour of males will depend on their contribution to rearing young and the defensibility of mates [12]. Males that invest heavily in paternal care typically mate with fewer females, as males cannot provide intensive paternal care to multiple litters. The defensibility of females is related to the size of female groups, the stability of those groups and the ranging behaviour of females [12]. For example, if females are highly dispersed then males are less able to secure and maintain access to several mates [12].

If males and females mate only with one partner, the population is considered to be monogamous. In most mammalian species, males mate with multiple females [24,141–143,145]. If males mate multiply but females do not, then the population is often classified as polygynous, whilst if both sexes mate multiply, it is considered to be promiscuous [12,24,141–143].

The level of mate competition varies according to the mating system adopted by a population [12,24,144,327]. The effects of mating system on the levels of mate competition between males before and/or after copulation are reasonably well characterised for mammalian species.

The OSR of populations with a monogamous mating system is predicted to be approximately 1:1. This means that neither sex is relatively 'limiting', so both sexes should experience approximately equal levels of mate competition. Competition for access to mates is typically lower in monogamous species than in species with other mating systems, so investment in pre-copulatory competition is generally low [148]. Members of both sexes are expected to have only one partner, so in strictly monogamous species there should be no sperm competition. However, extra-pair paternity has been detected in several 'monogamous' species, suggesting that sperm competition may occur [150]. Nevertheless, the level of sperm competition, and thus investment in post-copulatory competition, is expected to be lower in monogamous species than in polygynous or promiscuous species [358].

Males of polygynous species can maximise their reproductive fitness by securing and maintaining access to a group of females, so they compete primarily before copulation. The

high incidence of pre-copulatory competition in polygynous species often leads to the development of armaments. For example, in red deer (*Cervus elaphus*) males develop antlers that are used in overt, direct contests with other males [43]. As males typically defend access to several females, extra-group paternity is likely ([92], Section 3.1.3.1.). However, group males are expected to retain the majority of paternity without significant investment in post-copulatory competition.

In promiscuous species, females habitually mate multiply, so males typically experience high levels of sperm competition [117]. Levels of sperm competition are higher than in species with other mating systems [358,359] and males of promiscuous species have proportionately larger testes as a result [109,117]. Males may invest highly in pre-copulatory competition if the number of accessible females is low, for example because females are highly dispersed (discussed in e.g. [95]). However, investment in pre-copulatory competition is generally lower than in polygynous species.

3.1.4.2. Relatedness of competitors

3.1.4.2.1. Dispersal and the relatedness of males

Dispersal behaviour determines the relatedness of animals within a population, and thus whether related males are likely to compete for mates [196,223,311]. Males are more likely to compete with kin for mates if they remain together after sexual maturity. In most mammalian species, male kin disperse separately from the natal area and/or group (as in tigers (*Panthera tigris*) [360]) [24,141–143]. In such species, males disperse away from all known kin at or before sexual maturity, and males are likely to compete for mates with unrelated males. Male kin will remain together after sexual maturity if males are habitually philopatric or if males undergo natal dispersal as kin-groups (known as parallel dispersal) [143]. Males often remain philopatric where natal dispersal is female-biased [361]. Parallel dispersal of male kin is most often seen in species where brothers can collaborate to displace other males and secure access to females, as in the larger felids (e.g. lions [17] and cheetahs [362]).

Although males will compete among kin if they remain together, mate competition is unlikely to occur solely between related individuals, as females are expected to mate with extra-group males wherever possible [92].

3.1.4.2.2. Impact of male relatedness on investment in reproductive competition

Individuals maximise their reproductive fitness by ensuring that that their contribution of alleles to the next generation is as high as possible. This typically necessitates the adoption of reproductive strategies that allow individuals to outcompete rivals [363–365]. When males compete with unrelated animals for mates, their alleles may only be passed to the next generation 'directly' (i.e. by breeding to produce offspring), as they are unlikely to share a high proportion of alleles with competitors [117,218,219,365,366]. Under these circumstances, selection is expected to favour investment in 'selfish' reproductive strategies (i.e. a behaviour and/or trait which should maximise competitive ability against rivals) [365,367]. When competitors include relatives, males may gain fitness benefits both 'directly' and 'indirectly' because their alleles can be passed to the next generation by them or a related male with common alleles [117,218,219,365,366]. As males can benefit where kin breed, it may be adaptive to act less agonistically towards related competitors, provided that the benefits of doing so outweigh the costs [365]. It has therefore been hypothesised that males will invest relatively less in 'selfish' reproductive strategies when they compete with relatives [117,352,353,365,368]. .

Parker [117] used a modelling approach to investigate how relatedness of competitors may influence investment in sperm production under different levels of sperm competition. Three sperm-competition games were considered; (A) No information – males have no information about whether they are likely to face sperm competition, or whether it will occur between relatives, (B) Complete information - males have complete information about the risk of encountering sperm competition and their relatedness to competing males, (C) Information on relatives only - males have complete knowledge about risk of sperm competition with relatives, but not from unrelated males. In general, the results of the models from the three games suggested that, for a given level of sperm competition, males invested less in post-copulatory competition when competing with kin. Specifically, when males had either no or perfect information (games (A) and (B), respectively), ejaculate investment was expected to reduce with increasing probability of competitor relatedness. If information was only available on relatives, then the effect of relatedness on ejaculate investment was dependent on the risk of encountering sperm competition. If the overall risk of sperm competition was low, then males that competed with non-relatives (i.e. had no information) were expected to invest less in their ejaculate than males competing with kin. However, if the overall risk of sperm competition was high, then males with no information would invest more than males competing with kin [117]. Parker [117] recommended that the predictions of these models

be tested using comparative methods, but to my knowledge comparative testing has not been completed for any taxa.

The risks involved in competing with kin before copulation are distinct from those involved with competing after copulation. Males participating in pre-copulatory competitions are at risk of serious injury, or even death, during intra-male conflicts [29]. The loss of kin is therefore more likely in pre-copulatory contests. Male reproductive fitness may be significantly, negative affected by the loss of a member of a kin group, particularly in species where related males cooperate to secure females. For example, in male lions (*Panthera leo*), smaller coalitions of male lions are less likely to secure access to mates [17], so the lifetime reproductive success of all males in a coalition of brothers would be negatively impacted by the loss of a coalition member. Thus, any effect of relatedness on investment in reproductive competition may be more evident in pre-copulatory competition than in post-copulatory contests, which are relatively more subtle and associated with less aggression (see Section 3.1.4.1.).

There is intriguing, yet controversial, evidence from recent studies that relatedness may impact investment in pre-copulatory reproductive investment [352,353,365,368–370]. Carazo *et al*. [352] used the fruit fly *Drosophila melanogaster* as an experimental model to determine how male relatedness impacted the level of antagonism between male competitors and on the lifetime reproductive success of females. Courtship intensity and the level of male-male fighting were found to be lower when competition occurred between related males, rather than between non-kin [352]. This was regarded as good evidence that relatedness could influence investment in pre-copulatory reproductive competition. However, Carazo *et al*. [352] did not control for the social familiarity of males; related males were raised in one vial and were thus familiar, whilst unrelated males were not [368]. Hollis *et al*. [368] independently manipulated relatedness and familiarity in *D. melanogaster*. The study did not consider fighting or courtship intensity, but found that familiarity, rather than relatedness, was likely to explain the effects on female lifetime reproductive success found in Carazo *et al*. [352]. This, along with evidence from later studies [370], provides indirect evidence that relatedness may not be responsible for differences in investment in pre-copulatory competition [365]. Further experiments by Carazo *et al*. [353] and Martin and Long [365] confirmed that unrelated, unfamiliar animals fought more than related, familiar males. Martin and Long [365] considered the evidence for the impact of relatedness on investment reproductive competition to be tenuous. They presented preliminary evidence that indirect genetic effects (IGEs), rather than relatedness, may explain why levels of

antagonism were greater when unrelated males compete than between kin. They argued that related males are more genetically similar than non-kin and that, because behaviour is somewhat genetically determined, related males are thus more likely to express similar behavioural phenotypes. The combination of behavioural phenotypes in a group can influence the exhibition of different behaviours by group members. Indeed, the combination of behavioural phenotypes in a groups has been shown to influence level of aggression in *D. melanogaster* [371] and chemical signalling in *D. melanogaster* and *Mus musculus* [372]. Martin and Long [365] were able to predict the level of aggressiveness in a group from the combination of behavioural phenotypes for aggression in a group, which is evidence of an IGE. Although this preliminary evidence suggests that relatedness may be a poor predictor of the level of antagonism between males, further studies are still required to find further evidence for this hypothesis.

3.1.5. Aims of this study

In this study, I will assess how the relatedness of competing males influences investment in both pre- and post-copulatory competition. I expect that investment in post-copulatory competition would be lower where males compete primarily among kin. I also expected that the relatedness of males would influence investment in pre-copulatory competition, with males competing among kin likely to invest less in pre-copulatory competition. I will account for differing investment in reproductive competition by focussing on mammalian species that exhibit a promiscuous mating system.

3.2. Methods

3.2.1. Data collection

I collected data on mating system, dispersal behaviour, male and female body mass and testes mass from published sources.

To investigate the effect of male relatedness on investment in pre- and post-copulatory competition, it was necessary to minimise the variation in pre- and post-copulatory competition due to mating system. Data collection was therefore limited to promiscuous mammalian species (i.e. species in which both males and females mate with multiple individuals). Promiscuous species were identified during data collection for chapter 2 (see Section 2.2.1.). Where possible, mating system data were acquired from the same source as data on dispersal behaviour (see Section 2.2.1.).

Data on dispersal behaviour collected in chapter 2 (see Section 2.2.1.) were re-examined to determine the likelihood that males will compete with close kin for mates. The risk of intra-kin competition was considered 'high' if males were likely to remain with close male kin after sexual maturity. This included species for which there was evidence of extensive male philopatry (or female-biased dispersal (FBD)) and those in which males underwent natal dispersal as kin groups that remained stable. The risk of intra-kin competition was classified as 'low' if males dispersed separately from their natal group and/or area. Some classifications were less reliable than others. Classifications were regarded as most reliable if they were based on direct evidence of whether male kin remained together, and less reliable if they were inferred from the dispersal pattern for the species (e.g. when high male philopatry, and thus a high risk of intra-kin competition, was inferred because a species exhibited FBD). Direct evidence (i.e. direct observational data and/or genetic data) was available for 37 of the 44 species for which data on testes mass and male body mass were available. For the remaining 7 species, the relative risk of males competing with kin for paternity was inferred from the species' dispersal pattern. Analyses were conducted using the full dataset or only the most reliable data (hereafter the 'all data' and 'conservative data', respectively). Data from captive studies were excluded due to restrictions on natural dispersal behaviour.

Data on traits related to investment in sperm production (male body mass and paired testes mass) were collected from a number of reviews and primary resources (provided in Appendix 3). Where possible, data on these traits were acquired from the same study. Data were acquired for 44 species. For two of those species (*Brachyteles arachnoides* and *Cebus capucinus*) testes mass was approximated using data on testes volume (*B. arachnoides*) or testes size (i.e. length, width and depth) (*C. capucinus*). The equation for an ellipsoid was used to estimate testicular volume from size data (as in e.g. [373,374]). Testicular volume was converted to testes mass data using a density value of 1.04gm/ml (as calculated by [375] and used in e.g. [373,376,377]). All testes mass data was paired i.e. the sum of the mass of both testicles. Testes and male body mass data were only included if data were taken from sexually mature individuals.

Data on male and female body mass were used to determine the level of sexual size dimorphism (SSD). SSD data were available for 41 species in the 'all' dataset, and for 35 species in the 'conservative' dataset. SSD could not be included as a continuous variable, as this can introduce errors into PGLS analyses [378]. Instead, SSD was included as a discrete variable. The level of SSD was classified according to whether the level of SSD in a species was above a given threshold value or not. To do this, SSD was first calculated by determining

how much larger males were compared to females as a percentage of male mass (as in [379]). The data were then categorised to show whether males were at least 5%, 10%, 20% or 25% heavier than females. To ensure that SSD was calculated accurately, male and female body mass data were always acquired from the same sources. If body mass data on both sexes was not available from a common source, the species was not included in tests. All body mass data were from sexually mature individuals.

If multiple values were available for a given trait, average values were used. Data from literature searches were checked to ensure that all duplicate values were removed prior to the calculation of average values. Duplicate values could occur if data from a primary source was included in at least one review. If the data were not checked, then data from a single source could be included multiple times when calculating the average value, causing inaccuracies. A summary of the data used and relevant references are provided in Appendix 3.

3.2.2. Comparative methods

As outlined in Section 2.2.2. individual species cannot be considered truly independent as all species have a degree of shared evolutionary history. This non-independence must be accounted for in any analyses that includes multiple species [323,324] Here, the most recent best-estimate mammalian supertree, which is rooted and contains branch length data, [336] was used to control for evolutionary relatedness. For each analysis, the tree was pruned to include only the species within the dataset using the APE package [332] within R v. 3.1.0 [333]. Binomial names in the dataset were matched to those in the tree using information in the IUCN Red List dataset v. 2015-4 [334]. To improve the reliability of the analyses, polytomies in the tree were randomly resolved using APE [332].

3.2.2.1. Phylogenetic least squares regression tests

Phylogenetic generalised least squares (PGLS) regression analyses were used to test whether investment in post-copulatory competition was affected by male relatedness. This method employs a maximum likelihood (ML) framework to calculate Pagel's lambda (λ), which was used to determine the degree of phylogenetic dependence in the model. Values of λ close to 0 suggest no phylogenetic dependence. However, λ=1 suggests that traits evolve according to a Brownian model; inter-species differences in traits were proportional to the time since the species diverged [324]. PGLS analyses were conducted using the Caper package [380] in R v. 3.1.0 [333].

Phylogenetic dependence may not be apparent from λ when using relatively small datasets, such as those used this this study (n<50). In such cases, λ is approximated as 0 in PGLS analyses even where there is a phylogenetic signal. Likelihood profiles may be used to assess the validity of λ values. If λ is likely to be calculated in error, current convention dictates that a PGLS analysis should be approximated for both λ=0 and λ=1. This reveals whether an error in the calculation of λ is likely to influence the results of the PGLS analysis.

The use of residual measures (e.g. residual testes mass) in comparative tests can cause erroneous results [381]. For this reason, residual testes mass was not used as a measure of post-copulatory investment. Instead, the effect of body mass on testes mass was accounted for by including male body mass in models as an independent variable.

The assumptions of linear modelling must be met for the results of a PGLS test to be valid. Diagnostic plots of the residuals were created for each model to assess assumptions related to the distribution of the data. Continuous data (e.g. male body mass and testes mass) were log-transformed prior to inclusion in tests to improve normality.

3.2.2.2. BayesTraits

Maximum likelihood (ML) functions within the Discrete module of BayesTraits V. 2.0 [335] were used to examine whether investment in pre-copulatory competition was linked to the relatedness of male competitors. I used maximum likelihood tests (ML) (the methodology for which I previously outlined in Chapter 2) to test the associated hypotheses.

Males that competed with relatives were expected to invest less in pre-copulatory competition than males competing with non-kin. Here, I use the level of male-biased SSD as an approximation of investment in pre-copulatory competition, with greater levels of SSD associated with higher investment. If competitor relatedness influences investment in pre-copulatory competition as expected, then the risk of competing with kin should drive (or precede) changes in the level of SSD. For example, a transition from low to high risk of competing with kin is expected to occur before a reduction in SSD.

3.3. Results

3.3.1. The effect of relatedness of males on investment in post-copulatory competition

In this section, I explore whether investment in sperm production is influenced by the risk that males will compete with close kin for mates. After controlling for the effect of body mass, a significant proportion of the variance in testes mass was accounted for by the risk that

sperm competition will occur between kin, regardless of the dataset used (all data: $F_{(d.f.)}$= $41.29_{(2,41)}$ P<0.001, R^2= 0.668, conservative data: $F_{(d.f.)}$= $46.37_{(2,34)}$ P<0.001, R^2= 0.732). The full results for each PGLS model are presented in Table 3.1. Contrary to predictions based on kin selection, the results suggest that investment in sperm production is higher among species where males are more likely to compete with kin for mates.

Table 3.1. Results of phylogenetic least squares regression (PGLS) analyses investigating the effect of intra-kin mate competition between males on relative testes mass in promiscuous mammals. Risk of intra-kin competition has two levels; high and low.

Dataset	λ	Df	Variables	Estimate ± SE	T	P
All	0.582	2, 41	Intercept	-2.168 ± 0.942	-2.302	0.027
			Log(male mass)	0.665 ± 0.078	8.542	<0.001
			Risk of intra-kin competition (low)	-1.021 ± 0.467	-2.184	0.035
Conservative	0.419	2, 34	Intercept	-1.774 ± 0.867	-2.047	0.048
			Log(male mass)	0.634 ± 0.072	8.742	<0.001
			Risk of intra-kin competition (low)	-1.186 ± 0.485	-2.445	0.020

3.3.2. The effect of relatedness of males on investment in pre-copulatory competition

In this section I will investigate whether the relatedness of male competitors influences SSD, specifically whether higher relatedness causes lower SSD. The results generated using the 'all' and 'conservative' datasets in ML tests are provided in Tables 3.2 and 3.3, respectively.

Table 3.2. Results of maximum likelihood (ML) tests conducted using data from the 'all' dataset to investigate evidence of correlated evolution between the relative risk of intra-kin competition between males and sexual size dimorphism (SSD). Dependent and independent Lh refer to the log-likelihood values produced in BayesTraits for the dependent and independent models, respectively. Model of evolution indicates how traits are predicted to have evolved, 'dependent' suggests that the evolution of traits was correlated, 'independent' suggests that the traits evolved independently.

Level of SSD	Dependent Lh	Independent Lh	ML value	Df	P	Model of evolution
5%	-35.243	-37.387	4.289	4	0.368	Independent
10%	-39.268	-39.592	0.646	4	0.958	Independent
20%	-38.279	-45.065	13.572	4	0.009	Dependent
25%	-34.998	-41.012	12.029	4	0.017	Dependent

Table 3.3. Results of maximum likelihood (ML) tests conducted using data from the 'conservative' dataset to investigate evidence of correlated evolution between the relative risk of intra-kin competition between males and sexual size dimorphism (SSD). Dependent and independent Lh refer to the log-likelihood values produced in BayesTraits for the dependent and independent models, respectively. Model of evolution indicates how traits are predicted to have evolved, 'dependent' suggests that the evolution of traits was correlated, 'independent' suggests that the traits evolved independently.

Level of SSD	Dependent Lh	Independent Lh	ML value	Df	P	Model of evolution
5%	-31.510	-32.140	1.260	4	0.868	Independent
10%	-30.201	-32.029	3.656	4	0.455	Independent
20%	-34.145	-38.786	9.283	4	0.054	Independent
25%	-30.689	-36.045	10.712	4	0.030	Dependent

As is evident in Table 3.2., there was no evidence for correlated (dependent) evolution at lower levels of SSD (i.e. 5% and 10%) when using the 'all' dataset. However, there was evidence of dependent evolution between the likelihood of intra-kin competition between males and higher levels of SSD (i.e. 20% and 25% SSD). There was only evidence of dependent evolution between the risk that mate competition would occur between kin and SSD when SSD was classified using a threshold of 25% (Table 3.3.).

The results of ML tests can show whether the evolution of a pair of traits is linked ('dependent evolution') or not ('independent evolution'). However, ML tests cannot show how exactly traits are related when there is evidence of dependent evolution. I therefore used ancestral state and transition rate values from models assuming dependent evolution to investigate the nature of apparent relationships between traits (i.e. where there was evidence of dependent evolution between traits from ML tests). I present ancestral state and transition rate values for the relationships evident when using the 'all' and 'conservative' to construct models in Figures 3.1. and 3.2., respectively.

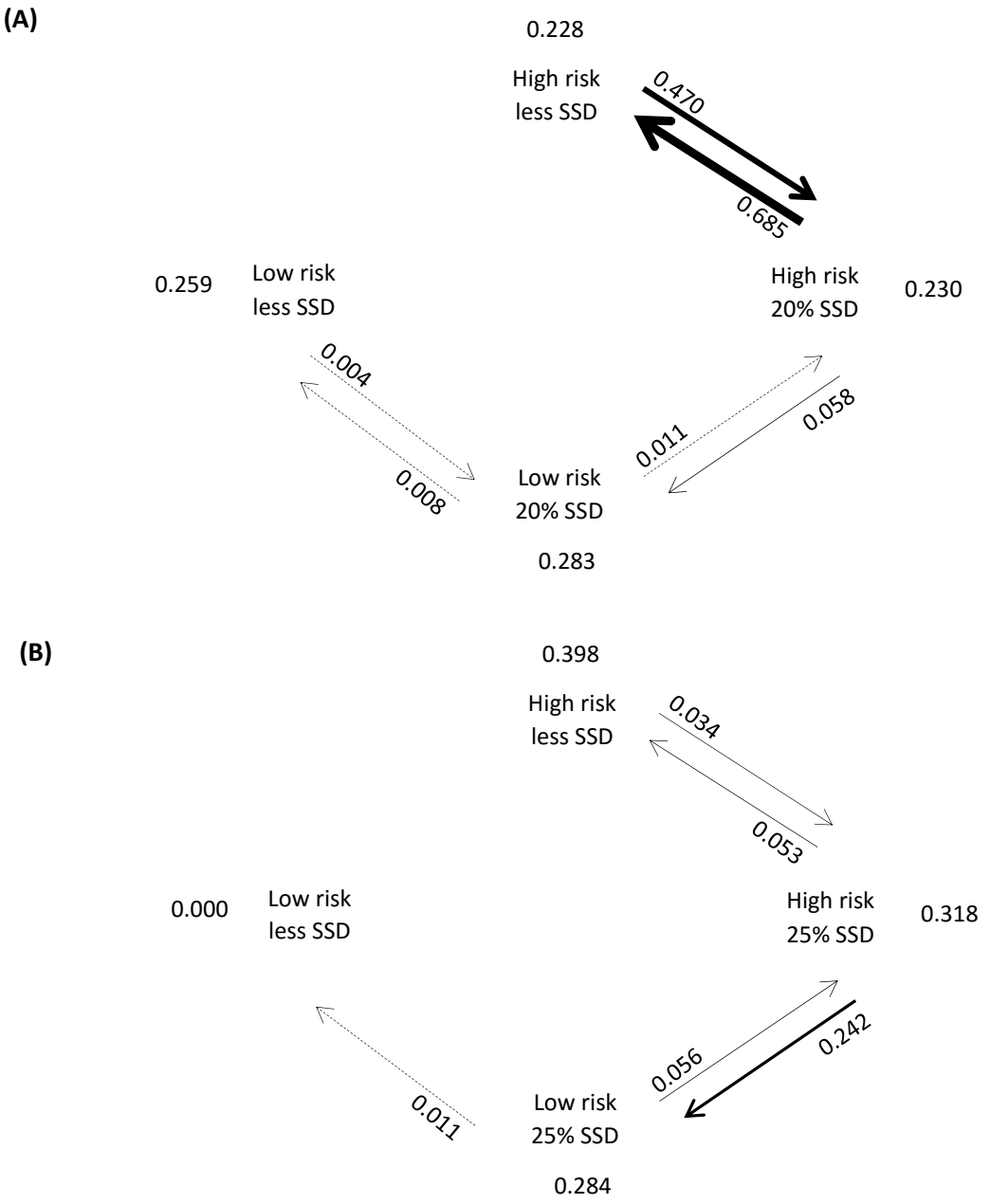

Figure 3.1. Coevolution between the likelihood of males competing with relatives and sexual size dimorphism (SSD) evident when using the 'all' dataset to construct models (A) model generated when SSD is at least 20% (B) model generated when SSD is at least 25%. 'High risk' indicates that males are likely to compete with relatives whilst 'low risk' indicates that they are unlikely to compete with relatives. SSD is classified according to whether males were at least 20% or 25% heavier than females (20/25% SSD, respectively) or not ('less SSD' in each case). Arrows represent transitions between states, and are scaled to represent the transition rate, which is provided as text on the respective arrow. Transition rates of below 0.05 are represented with dotted lines, and arrows are absent if a transition rate is zero. Ancestral state values are provided beside trait combinations.

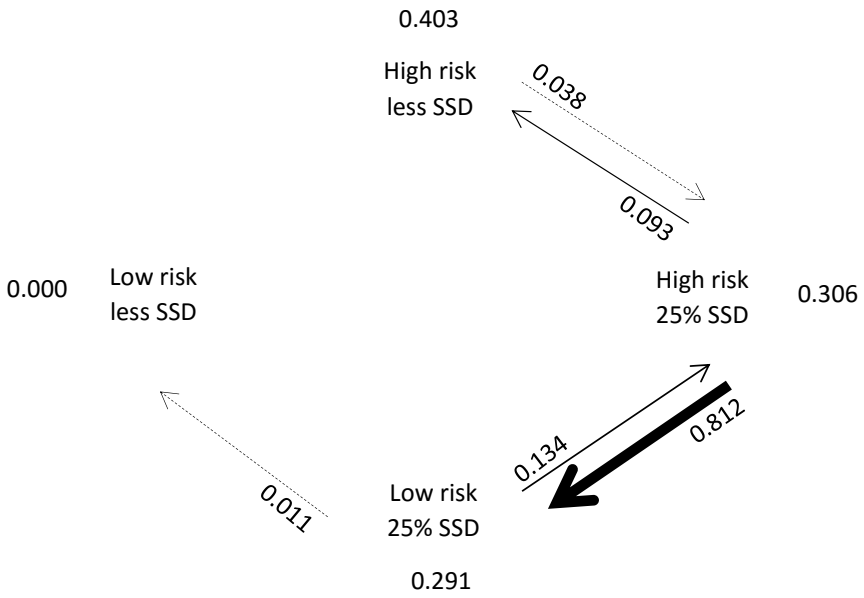

Figure 3.2. Coevolution between the likelihood of males competing with relatives and sexual size dimorphism (SSD) evident when using the 'conservative' dataset to generate models. 'High risk' indicates that males are likely to compete with relatives whilst 'low risk' indicates that they are unlikely to compete with relatives. SSD is classified according to whether males were at 25% heavier than females (25% SSD) or not ('less SSD'). Arrows represent transitions between states, and are scaled to represent the transition rate, which is provided as text on the respective arrow. Transition rates of below 0.05 are represented with dotted lines, and arrows are absent if a transition rate is zero. Ancestral state values are provided beside trait combinations.

Males are expected to invest less in pre-copulatory competition when they compete with relatives than when competing with non-kin. If this is the case, then transitions in the relatedness of competitors (i.e. the relative risk of competing with kin) should precede transitions in SSD. The order of transitions can only be established if the ancestral state can be identified.

Although ML tests indicated that there was a link between 20% SSD and the risk of intra-kin competition, all trait combinations (e.g. high risk, 20% SSD) were approximately equally likely to be the ancestral state (see ancestral state probabilities in Figure 3.1A). As the ancestral state could not be identified, it was not possible to deduce the nature of the association between 20% SSD and risk of intra-kin competition between males.

In models where the threshold values of SSD was set to 25%, the most likely ancestral state was 'high risk, less SSD' for both datasets used (see Figures 3.1A and 3.2). This suggests that in the most recent common ancestor of these species, males were less than 25% larger than females and were likely to compete with kin for mates. Given the transition rate values, a transition in SSD is seemingly more likely than a change in the relative risk of intra-kin competition from the ancestral state. This is contrary to expectations, as it suggests that the risk of intra-kin competition differs according to the level of SSD. Specifically, an increase in SSD (such that males become at least 25% heavier than females) leads to a reduction in the risk that related males will compete for paternity in promiscuous mammals (Figures 3.1 and 3.2).

3.4. Discussion

The primary aim of this chapter was to investigate how the relatedness of males, determined by dispersal behaviour, influenced investment in reproductive competition. In particular, I considered whether investment in pre- and post-copulatory competition was likely to be reduced when the risk of intra-kin competition between males of promiscuous mammalian species was high. In this section, I discuss the implications of the results before summarising the conclusions which can be drawn from this study and detailing how this area could be progressed in future.

3.4.1. Risk of intra-kin competition and investment in sperm production

Based on the results of models in Parker [117], ejaculate investment was expected to be lower where sperm competition was likely to occur between kin. After controlling for the effect of body mass on testes mass, I found a significant link between testes mass and male competitor relatedness. Specifically, testes mass was greater in promiscuous mammalian species where males were more likely to compete with same-sex relatives. This suggests that males invest more in pre-copulatory competition when the risk of intra-kin competition are high.

I restricted the analyses to promiscuous species in an attempt to minimise inter-species variation in the risk and level of sperm competition. However, there can be variation within a mating system due to several factors, potentially including relatedness. Indeed, when males compete with kin for mates, males are more likely to mate with a female that has already mated [117], potentially because female mating frequency is higher, thus the risk of sperm competition is greater when related males compete. Investment in ejaculates is expected to increase with the risk of sperm competition [107,117,132]. This could account for the

observation in this study that investment in ejaculates was higher when males were more likely to compete with relatives in promiscuous mammals. To investigate whether this was the case, it would be necessary to control for the level and risk of sperm competition more effectively than simply accounting for differences due to mating system. However, data on factors that could enable an estimation of the levels and risk of sperm competition, such as female mating frequency, are rare.

As the risk of sperm competition may not be comparable between species, the finding that investment in ejaculates is likely to be higher where males are more likely to compete with kin should not necessarily be considered evidence against the predictions of Parker [117]. Indeed, it is possible that, after controlling for the risk and level of sperm competition in each species, it would be evident that males invest less in their ejaculates when competing with kin. However, the results of studies using individual species suggest that the relatedness of competing males may not influence investment in ejaculates. In each of the three studies completed to date (on Australian crickets (*Teleogryllus oceanicus*) [382], house mice (*Mus musculus domesticus*) [383] and bank voles (*Myodes glareolus*) [312]), there was no evidence that males adjust investment in ejaculates according to the relatedness of competitors. Ramm and Stockley [383] suggested that the lack of kin-biased behaviour may be related to kin recognition in *M. musculus domesticus*. Mice use major urinary proteins (MUPs) to identify kin, but may share MUP haplotypes with siblings [210]. Males may identify brothers with the same MUP haplotype as 'self' and other brothers as 'non-relative'. This could account for the lack of an effect of competitor relatedness on investment in ejaculates [383]. The dispersal behaviour of the focal species may also account for the findings of these studies. In *M. glareolus*, males disperse separately from the natal group [23,318] and may be unlikely to encounter sperm competition with a related individual. Where competition with kin is unlikely in natural populations, males may be unlikely to vary sperm allocation according to the potential relatedness of competitors [312,383]. Further studies are necessary to investigate whether there is evidence that males invest less in sperm competition when competing with relatives in other species, perhaps those with different mechanisms for kin recognition or dispersal pattern than those previously studies.

3.4.2. Risk of intra-kin competition and investment in pre-copulatory competition

Selection for escalated contest competition, and hence high SSD, was expected to be less intense where males compete primarily among relatives (see Section 3.1.3.1.). SSD was thus expected to be dependent on male relatedness, with a high risk of intra-kin competition

causing low levels of male-biased SSD. Consistent with this prediction, I found evidence of correlated evolution between investment in pre-copulatory competition and risk of intra-kin competition, but only where SSD was classified according to the presence/absence of at least 20 or 25% SSD. However, contrary to expectations, I found that evolutionary transitions in pre-copulatory investment (i.e. SSD) were likely to occur *before* transitions in competitor relatedness. Specifically, the evolution of an increased investment in pre-copulatory competition (such that males become at least 25% larger than females) appears to favour the evolution of reduced intra-kin competition. Overall, these results suggest that male relatedness does not influence investment in pre-copulatory competition, in accordance with the results of previous studies on *D. melanogaster* [365].

Although there is no evidence that investment in pre-copulatory competition is influenced by male relatedness, the results of this study are somewhat consistent with kin selection theory [218,219]. The results indicate that increases in pre-copulatory competition may cause a reduction in the risk that males will compete with kin. A reduction in the risk of intra-kin competition could be achieved with a change in male dispersal behaviour. For example, if the risk of intra-kin competition was high because males are philopatric, the risk of reproductive competition occurring between kin would be reduced if male dispersal behaviour changed so that males instead dispersed separately from the natal group (see Sections 3.1.4.2.1. and 3.2.1). High levels of pre-copulatory competition are typically associated with a high risk of injury [29]. If males that are likely to compete with kin adopt a reproductive strategy which includes high levels of pre-copulatory competition, they risk seriously injuring their relatives. By altering dispersal behaviour to reduce the likelihood of competing with kin where there are high levels of pre-copulatory competition, males could minimise the chances of seriously injuring relatives or excluding them from breeding. This strategy could allow males to maximise reproductive fitness by gaining as many direct fitness benefits as possible without limiting the potential for indirect fitness benefits. However, there is only evidence that males are more likely to minimise the risk of competing with kin where males are at least 25% larger than females (i.e. extremely high levels of pre-copulatory investment). This could indicate that kin selection does not influence male dispersal behaviour unless there are extremely high levels of pre-copulatory investment.

High levels of male-biased SSD are more typically associated with polygynous than promiscuous species ([346]; Section 3.1.4.1.). Despite this, some promiscuous species in this study exhibited high levels of SSD. Species were only classified as promiscuous if there was clear evidence of multiple mating by both males and females in the primary literature

(Section 2.2.1.). However, data on male and female body mass was not typically available from the same studies which contained information on the mating system of a species. It is therefore possible that data on size dimorphism was taken from a population or populations of a species which exhibited polygyny rather than promiscuity. Mating system has been shown to vary between populations and possibly between years in mammal species [327,329]. Mating system may vary according to environmental factors (e.g. habitat type) and/or differences in population structure (e.g. population density and sex-ratio) [329]. Species may also have been misclassified in the literature. For example, behavioural observations of the dusky footed woodrat (*Neotoma fuscipes*) suggested that the species was polygynous, whilst genetic tests indicated that it is likely to be promiscuous [329]. Where possible, I gained several references to increase the reliability of mating system classifications. However, it is possible that some species are incorrectly classified as promiscuous. Similarly, data on SSD may not be representative of the species. I attempted to minimise this risk by collecting data from multiple studies, where possible. Without further studies on the species included in this study, it is not possible to determine the extent to which the results may have been affected by any misclassifications. Another important consideration is that the dataset used in this study included only a fraction of mammalian species, so it may not be representative of this species group [145]. Future studies using larger sample sizes would be necessary to determine whether this was the case.

3.5. Conclusions

I found that, contrary to expectations, relative testes size was significantly higher in promiscuous mammals where reproductive competition between males was likely to occur between relatives. The result may differ from expectations because the level of sperm competition was greater where risk of intra-kin competition was high, even though the species all exhibited similar mating systems. I also found no evidence that investment in pre-copulatory competition was lower when males compete with kin. The results did suggest that males may attempt to minimise the risk of competing with kin following increases in the investment in (and thus likely the level of) pre-copulatory competition. This may be evidence of kin selection, as it could indicate that males attempt to avoid seriously injuring kin, but not non-kin.

Chapter 4: Maternal effects on life history traits in bank voles (*Myodes glareolus*)

Abstract

A maternal effect consists of an impact of the mother on offspring phenotype which is not solely genetically determined. Social competition may influence maternal effects on several traits including offspring body size, offspring reproductive traits and litter sex ratio. I used bank voles (*Myodes glareolus*) as an experimental model to investigate the impact of social competition on maternal effects. I used social cues to simulate 'high' and 'low' levels of social competition in female bank voles. Maternal effects may be mediated by the action of maternal hormones, including corticosterone. I therefore assessed levels of corticosterone in female bank voles in each treatment group before pregnancy. There was no evidence of a difference between treatment groups, possibly due to problems with the assay procedure. I found no evidence of a maternal effect on traits related to offspring body size, or in the masses of testes and seminal vesicles. However, the mass of epididymides and daily rates of sperm production were relatively increased in males born in the high competition group. This is evidence of an adaptive maternal effect on male reproductive traits; both traits are associated with increased ejaculate investment, consistent with greater levels of reproductive competition expected in 'high' competition environments. Lastly, I detected a significant difference in litter sex ratio between groups. Females in the high competition treatment group produced more female-biased litters, inconsistent with expectations based on the local resource competition (LRC) hypothesis. The results of this study provide valuable insights about the action of maternal effects due to social competition on life-history traits in mammalian species.

4.1. Introduction

4.1.1. Maternal effects

The definition of maternal effects has varied considerably in the 70 years since they were first described [246,247]. Broadly though, maternal effects may be defined as any influence of the mother on the phenotype of offspring that is not solely genetically determined [248]. Most of the maternal effects detected in early studies had a negative impact on offspring reproductive success (i.e. were maladaptive) [249,384–386]. In many early studies, maternal effects resulted due to differences in resource accessibility between mothers. Females that had less access to resources produced young that were smaller, took longer to mature and

had lower reproductive success compared to other females [249,253,384–386]. However, maternal effects are increasingly recognised as potentially adaptive mechanisms by which mothers can 'match' offspring phenotype to their local environment to maximise offspring reproductive success [249,251–255].

4.1.2. Maternal effects and the social environment

The 'social environment' of an individual is largely determined by density, composition and social structure of populations. These factors each influence the nature of social interactions between individuals. For example, when population density is high, competition for the finite resources in the area will be increased, causing higher rates of aggressive encounters [154,173,387–390]. Previous studies, which have primarily been conducted using guinea pigs (*Cavia porcellus*), have shown that the social environment experienced by the mother during pregnancy can cause maternal effects [385,386,391]. Maternal effects due to the prenatal social environment have been identified in several traits related to offspring competitive ability and likely reproductive success, including body mass [253,263,269], sex [249,392,393] and investment in reproductive traits [273,310,394].

4.1.2.1. Maternal hormones as a potential mediator of maternal effects

Maternal hormones have been proposed as dynamic mediators of maternal effects in species across taxa including birds [276,395,396], reptiles [261,397–399] and mammals [251,287,400]. In oviparous species, maternal hormones are deposited in eggs where they can act on young to cause a maternal effect [276]. By contrast, in viviparous mammals, maternal hormones can pass from the mother to offspring *in utero* via the placenta [251]. The levels of maternal hormones, such as cortisol and testosterone, and of other molecules, including glucose, have been shown to be related to maternal effects on several traits in mammalian species [251,254,384–387,391].

Most studies investigating relationships between maternal hormones and maternal effects have focussed on glucocorticoids (i.e. stress hormones) [384–386,391]. Some such studies used non-social stressors, such as immobilisation, to cause elevated levels of glucocorticoids [385,386,401]. Whilst such stimuli have been shown to cause maternal effects, their relevance to animal ecology and behaviour has been called in to question [385,386]. Other studies investigated how factors related to the social environment could influence the levels of maternal stress hormones, and thus cause maternal effects [251,384–387,391]. For example, when population density is high individuals are more likely to experience competition for resources. This could cause levels of glucocorticoids (e.g. cortisol) in the

bloodstream to be relatively increased in animals in high density habitats [251,384–387,391]. Levels of stress hormones may be elevated in response to high population density for several reasons including a relatively high level of antagonistic interactions with conspecifics and exclusion from resources required for survival and/or reproduction [251,387].

In some studies considering how maternal hormones can cause maternal effects, biologically relevant social cues are manipulated to simulate different levels of population density/social competition [385–387]. One of the factors that varies with population density is social group stability, with higher instability associated with greater levels of population density [385,386]. In various studies by Sachser, Kaiser and colleagues, the stability of guinea pig (*Cavia porcellus*) social groups were manipulated under laboratory conditions to simulate different levels of population density (reviewed in [385,386,402]). This has resulted in the expression of maternal effects on several traits including those related to responses to stress and reproduction [385,386,402]. The impact of maternal hormones on maternal effects has also recently been shown in field conditions. Dantzer *et al*. [387] used playback vocalisations to simulate different levels of density in populations of American red squirrels (*Tamiasciurus hudsonicus*). Levels of corticosterone were found to be greater in pregnant females in 'high density' groups compared to those in 'low density' conditions. Offspring growth rate was found to be associated with levels of maternal corticosterone. The effect on growth rate could be replicated by artificially increasing glucocorticoid levels in pregnant females, showing that the maternal effect was a direct result of maternal hormones [387]

4.1.2.2. Maternal effects due to high density conditions

Maternal effects are considered to be adaptive if they cause a phenotypic change which confers an advantage on offspring reproductive success in a given set of environmental conditions [280,387,391,403]. High population density can cause maternal effects in several traits, and there is evidence that many of these may be adaptive [81,258,403–405].

4.1.2.2.1. Maternal effects on offspring size and number

When population density is high, individuals will experience greater levels of competition to access resources, including mates [24,141]. The ability of animals to acquire resources when competition is high can be related to body size, with larger individuals typically having an advantage [47,307]. Maternal effects can increase offspring body size by causing greater offspring birth weight [257], elevated offspring growth rates [263,264,267,271] and/or reducing litter size [275,406,407].

Females may manipulate litter/clutch size (i.e. number of offspring in one reproductive attempt) as a means of modifying parental investment. For example, female arctic foxes (*Alopex lagopus*) wean larger litters when resource availability is high and they can afford to invest more in litters [408]. However, changes in litter size do not necessarily indicate a difference in overall prenatal investment. In their classic model, Smith and Fretwell [406] proposed that there was a balance between offspring size and number. Specifically, they predicted that whilst increased investment would lead to the production of fewer pups overall, the pups produced would be 'fitter' (i.e. more able to survive and reproduce) as they would be relatively larger.

In mammals, mothers can alter the number of offspring produced either by reabsorbing young in the womb and/or by rejecting some young postnatally [249,408,409]. There is evidence in support of the Smith-Fretwell model in several mammalian species [410–412], indicating that maternal effects on offspring body mass can occur due to changes in litter size. Some of the best evidence for the model to date comes from observations of post-natal maternal care. Individuals born in relatively large litters are expected to acquire lower levels of maternal provisioning, possibly including a smaller share of milk, typically resulting in such individuals being relatively small [263]. Thus, there is a clear trade-off between the size and number of pups [263]. Although maternal effects on litter size could act adaptively on offspring body mass in high density conditions, there is relatively little evidence for variation in litter size according to population density in mammalian species studied to date [411,413].

Offspring body mass may also be affected by maternal effects on growth rates [253,263,267,268,270,271,276,387]. Maternal effects on growth rate are often associated with resource accessibility during pregnancy, which is often associated with population density. The offspring of females with relatively low access to resources are typically smaller, grow more slowly and mature at a more advanced age than the offspring of conspecific females, as seen in wild savannah baboons (*Papio cynocephalus*) [271] and harbour seals (*Phoca vitulina*) [267]. Such maladaptive maternal effects can persist through generations, causing long term negative effects on offspring fitness [253]. However, not all maternal effects in high density conditions are maladaptive. Dantzer *et al*. [387] found that female *T. hudsonicus* presented with cues suggesting high population density produced faster growing offspring, even in the absence of additional resources. Faster-growing offspring matured earlier and were more able to acquire access to resources crucial for overwinter survival.

Maternal effects on offspring body size (due to alterations in litter size, growth rate or some other factor) should be evident when offspring become independent of mothers (i.e. weaning in mammals) [263,267] and are usually sustained into adulthood [253].

4.1.2.2.2. Maternal effects on offspring reproductive traits

Maternal effects on reproductive traits are relatively understudied compared to maternal effects on most other traits. However, there is evidence that maternal effects can influence reproductive traits in both males [81,255,273,279,310,414] and females [255,310], although most studies to date have focussed on males. There is some evidence that population density and the respective social competition can influence maternal effects on reproductive traits in mammalian species. A recent study on root voles (*Microtus oeconomus*) provided evidence of maladaptive maternal effects resulting from high population density. Specifically, offspring of mothers in high density environments were reproductively suppressed. Reproductive suppression in young was found to persist regardless of subsequent changes in population density [255]. The findings from that study were consistent with those from other vole species, and suggest that maternal effects on reproductive capacity could be the cause of oscillations in density observed in vole populations [260].

Studies on the guinea pig (*C. porcellus*) have instead found evidence of adaptive maternal effects on reproductive traits. Pregnant guinea pigs exposed to cues indicating high population density produce masculinised female and feminised male offspring [384,385]. Masculinised females have relatively poor fertility, but are more likely to secure more dominant social positions and to secure access to resources. This means that although they are reproductively impaired, their reproductive success is likely to be relatively high compared to other females under high density habitats [384,385]. Feminisation caused males to reach sexual maturity at a slower pace than other males. Whilst such an effect could be maladaptive in some species (Section 4.1.2.2.1), in *C. porcellus* it may confer an advantage in high density conditions [384,385]. When population density is high, male *C. porcellus* will compete heavily for access to mates, and younger males are likely to be outcompeted by older, more dominant males. Thus, challenging older males at a young age is unlikely to improve reproductive success. By causing a delay in sexual maturity, the feminisation of males ensures that individuals are able to avoid competition with more dominant males at an early age [384,385]. The findings from *C. porcellus* highlight the importance of considering maternal effects in an appropriate ecological context to ensure that the adaptive values of traits are not misinterpreted.

4.1.2.2.3. Maternal effects on offspring sex

Maternal fitness can be influenced by sex of offspring they rear [415,416]. The relative reproductive value of male and female offspring may vary according to the social structure of populations [416,417] and environmental conditions [418]. Thus, some conditions are likely to favour the production of one sex over the other [417,418]. Females can modify the sex ratios of litters/clutches, although the mechanism for altering sex ratio is poorly understood where offspring sex is chromosomally determined [266,392,419].

The local resource competition (LRC) hypothesis predicts that the relative production of each sex will vary according to the probability of competition between members of the philopatric sex and mothers [416,418,420]. Philopatry causes the number of animals, and thus the level of competition, in an area to be increased. Females in high density environments are unlikely to benefit from high levels of production of the philopatric sex, as it would cause a further increase in the levels of competition in the area in which the mother is resident. Instead, females in high density environments are likely to benefit from relatively greater levels of production of the more dispersive sex [416,418,420]. In mammals, females are typically the more philopatric sex [24,141–143,145]. A recent cross-fostering experiment on tammar wallabies (*Macropus eugenii*) found some evidence for the LRC hypothesis; rearing more daughters reduced future reproductive fitness of mothers [416]. Further studies are required to determine whether there is support for the LRC hypothesis in other mammalian species.

4.1.3. Maternal effects and bank voles

In this study I use bank voles (*Myodes glareolus*) as an experimental model to investigate how population density, specifically the associated levels of social competition, may influence maternal effects on offspring body size, offspring reproductive traits and litter sex ratio.

4.1.3.1. The bank vole

The bank vole (*Myodes glareolus*) is a generalist rodent which is commonly resident across Europe [284]. Both males and females exhibit territoriality during the breeding season. The home ranges of individuals of different sexes overlap, enabling animals of each sex to mate with multiple partners, thus making the species promiscuous [23,293,294,311,394]. Members of both sexes may disperse from the natal area. but natal dispersal propensity is generally higher in males [320].

4.1.3.2. Previous studies of maternal effects on bank voles

Relatively few studies have examined maternal effects in *M. glareolus.* However, maternal effects on some traits have been detected in response to exposure to social cues [310,401] and non-social stressors [401]. Marchlewska-Koj *et al.* [401] found that females exposed to cues indicating high density and non-social stressors produced more aggressive offspring, although the effect was particularly pronounced in males. Similarly, Marchlewska-Koj *et al*. [310] found that exposure to cues indicating high density caused reduced attractiveness and reproductive suppression in both males and females, but had no effect on weaning weight. These studies provide some insight into how maternal effects may act given exposure to different levels of population density. However, further studies are required to gain a more comprehensive understanding of maternal effects both in bank voles, and in mammals more generally.

4.1.4. Aims of the study

In this chapter, I investigate whether female bank voles subject to cues indicating high levels of social competition (which would be expected under relatively increased levels of population density) exhibit relatively increased levels of corticosterone, and whether the cues are sufficient to cause maternal effects. I specifically test for maternal effects on litter size, offspring growth rate, weaning weight, reproductive traits in males and litter sex ratio. I expect that any maternal effects on offspring size and litter sex ratio will be adaptive, with mothers in 'high density' conditions producing larger young, and lower numbers of females. However, given previous experiments on vole species, including *M. glareolus*, I predict that high density cues will cause reproductive suppression in males.

4.2. Methods

This study was completed using bank voles (*Myodes glareolus*). All work was conducted under the authority of The University of Liverpool's Animal Welfare Committee. The research adhered to the guidelines for the use of animals in research outlined by the Study of Animal Behaviour/Animal Behaviour Society [421]. The work was in accordance with UK Home Office code for the housing and care of animals bred, supplied or used for research and EU directive 2010/63/EU.

4.2.1. Animal health

Bank voles may develop diabetes both in the wild and in captive populations ([422]; present study). I tested all individuals in the captive population for diabetes. In order to determine

whether an individual was diabetic, I tested for the presence of glucose in a urine sample using Diastix™ reagent strips for urinalysis (Bayer, Germany). Only healthy animals were included in tests. Any animals in the captive population that exhibited symptoms of diabetes (e.g. consistent polyuria [423]) or any other disease were culled. I managed breeding in the captive population to maximise genetic diversity.

4.2.2. General housing conditions

The floor of each cage was covered in substrate (Corn Cob Absorb 10/14 substrate). Each cage also contained paper-wool nest material and a cardboard tube or box for shelter and enrichment. Food and water were provided *ad libitum* (LabDiet 5002 Certified Rodent Diet, Purina Mills, USA). Animals were maintained at a temperature of 20 ± 1^{O}C and on a reversed photoperiod (dark 8hr; light 16hr; white lights on 1700hr). All contact with animals occurred during in the dark period under red lighting.

4.2.3. Adult bank voles included in the experiment

4.2.3.1. Adult females

All adult females used in the study were bred in the laboratory and were first- and second-generation descendants of wild-caught bank voles trapped in Cheshire (UK). The social experience of individuals was comparable before tests; all adult females were unmated and had been singly housed since weaning. Females were 8-10 months old at the beginning of the study period. Adult females were housed singly in MB1 cages (45 x 28 x 13cm, North Kent Plastic cages Ltd., UK) in the same room both before and during the study.

4.2.3.2. Adult males

Adult males used in tests were either lab-bred or wild-caught in Cheshire (UK). Wild-caught males were maintained in the lab for at least 4 weeks before being included in the study. The ages of wild-caught males were unknown,

All males were singly housed in M3 cages (48 x 15 x 13cm, North Kent Plastic cages Ltd., UK) before the study. Males used for breeding were housed with females for 14 days during the study.

4.2.4. Treatment regime: manipulating the social experience of adult female bank voles

The study was completed in two blocks. The methods used to produce animals and to divide individuals between treatment groups were identical in each block. The treatment regime used is modified from that used in Ramm & Stockley [424] and Lemaître *et al*. [102].

4.2.4.1. Division of animals between treatment groups

Sixteen adult females were included in each study block, although the 16 subjects used differed between blocks. Animals in each block were divided equally between two treatment groups; high and low competition. The age and relatedness of females were balanced across the treatment groups. The ages of subjects ranged between 8 and 10 months, by balancing the ages between groups I minimised the risk of any difference between treatment groups being due to age. Some of the adult subjects used in the study were related. I divided related subjects between the two treatment groups as equally as possible to avoid any effect due to relatedness.

Adult males were not subject to a treatment. However, males were used for breeding and male odours were presented to females at certain points of the study. For this reason, males were divided according to treatment group. The origin of males (i.e. lab-bred or wild-caught) and the age and relatedness of lab-bred males was balanced between treatment groups. The lab-bred males failed to breed in the first study block, so only wild-caught males were used for breeding in the second study block in an attempt to maximise the number of litters produced.

4.2.4.2. Manipulation of social experience; odour cues and direct exposures

Olfaction is the primary sensory modality of rodents [424], and individuals are able to identify conspecifics using odour cues [425]. Thus, scent samples and direct inter-individual encounters were used to manipulate the social experience of adult females. Females in the 'high competition' group were exposed to (i.e. encountered and received scent samples from) three females throughout the study, whilst those in the 'low competition' group were only exposed to one other female.

As all the animals were housed in the same room, there was a possibility that each female would hear and/or smell other individuals. To minimise exposure to females not due to the treatment provided, I placed cages containing individual females in high sided indoor enclosures (width: 60cm length: 60cm, depth: 78cm). Two cages were placed in each of eight enclosures to minimise the possibility of any difference in to housing conditions between treatment groups. The pair of females in each enclosure were from the same treatment group. Females in the low competition group were only exposed to the other female in the same enclosure throughout the study period. Females in the high competition group were exposed to the other female in the same enclosure and a pair of females in the enclosure in the same row as theirs. The positions of females in different treatment groups in the eight

enclosures are shown in Figure 4.1. Each enclosure was positioned on the floor, and all enclosures were clustered together (as in Figure 4.1).

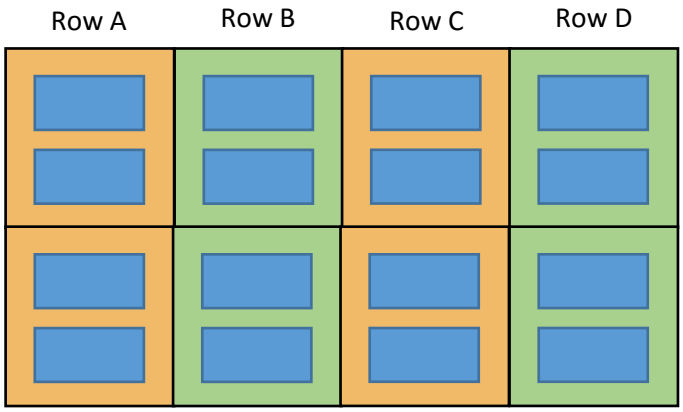

Figure 4.1. Positions of females in each treatment group in eight indoor high-sided enclosures. High-sided enclosures are outlined in black. Cages are depicted in blue. Row A-D refer to rows of enclosures. Rows containing the cages of high competition females are coloured orange, and those containing the cages of low competition females are depicted in green. This set up was the same in each block.

Females received scent samples from conspecifics over a period of 12-13 weeks, depending on the timing of parturition of offspring (Section 4.2.4.2.1.). The treatment ended when all offspring were weaned. By contrast, direct exposures only occurred during the first six weeks of the study; before the females were allowed to mate. The schedules and methodologies used for providing scent samples from females and for allowing direct exposures are detailed in Sections 4.2.4.2.1 and 4.2.4.2.2., respectively.

4.2.4.2.1. Exposure to scent samples from conspecific females

The odour exposure regime was designed to simulate both a different number of female competitors and a different frequency of intrusions.

Scent samples consisted of ~25g of soiled sediment. The sample was removed from each cage using a plastic weighing boat. Each female was assigned a different weighing boat, so there was no risk of cross-contamination between donors. The weighing boats were cleaned using 70% ethanol after each transfer. Scent samples were transferred directly from the donor cage to the recipient cage in the weighing boat. Where females received their own scent, their cage was both donor and recipient. To minimise the risk of cross-contamination between scent samples, I removed soiled sediment from the front of the donor cage and

presented the sample at the back of recipient cages. The order in which scent samples were presented to subjects in each 'row' of the enclosures and in different treatment groups were balanced as much as possible across treatment blocks.

Exposure to scent samples was performed in treatment blocks, each lasting two weeks and commencing on weeks 1, 3, 5, 7, 9, 11 and 13. The length of the last treatment block was shorter if all offspring were weaned before two weeks had passed. Scent samples were introduced to the cages of each female on days 5, 8, and 11 of each treatment block. Females only received an odour sample from one female on each of these days. Females in the high competition group (hereafter 'high competition females') received an odour from a conspecific in their row (see Figure 4.1) on each of these days. The order in which the scents of each of the conspecifics was presented was balanced across treatment blocks. Females in the low competition group (hereafter 'low competition females') only received a scent sample from a conspecific female on one of these days in each treatment block. On the other two days, low competition females received a scent sample taken from their own cage to control for cage handling effects across treatment groups. The day on which this scent sample was presented varied, but was balanced across treatment blocks. All females, received the odour from the conspecific in the same enclosure on the same day of a given treatment block (e.g. on day 8 all animals received a scent sample from the other animal in the same enclosure). I cleaned all cages on day 14 of the treatment block, so no scent samples remained in cages beyond the end of each treatment block.

Males were placed in female cages during one treatment block (that commencing on week 7). I used stored scent samples from females to prevent the transfer of male odours between cages during this period. I removed the required number of scent samples from each donor cage at the end of week 6 and stored each sample in a freezer at -2^{o}C until it was required. Each sample was thoroughly defrosted before use.

4.2.4.2.2. Direct exposures to conspecific females

Direct exposures occurred on day 1 of treatment blocks commencing on weeks 1, 3, 5 and 7. Individuals were only exposed to conspecifics from which they received scent samples. Thus, high competition females were allowed to encounter the 3 conspecifics in their row (see Figure 4.1), whilst low competition females were only able to encounter the other female in the same enclosure. In total there were 6 'encounter groups'; 2 sets of 4 high competition females, and 4 pairs of 2 low competition females.

Direct exposures occurred in an enclosure that was equally divided into four compartments (hereafter the 'encounter enclosure'). A schematic diagram of the encounter enclosure is shown in Figure 4.2. Individuals were able to see, hear and smell one another through circular 'windows' located 1cm from the enclosure floor. The windows comprised of circular holes (10cm in diameter) covered with a single layer of wire mesh. The mesh was sufficient to prevent subjects in different sections from injuring one another.

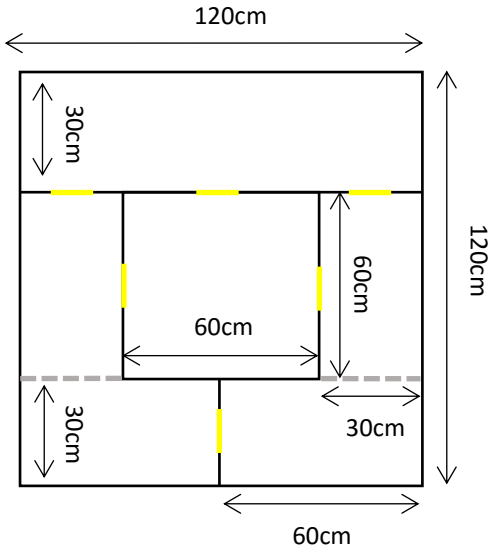

Figure 4.2. Schematic diagram of the encounter enclosure. Solid enclosure walls (78m high) are depicted using black lines. All outer walls are 1.5cm thick, and inner walls are 0.3cm thick. Dashed grey lines represent 'half walls', where there was only a solid wall over the upper 50cm of the enclosure. Adult females could move freely under half walls. Yellow lines indicate the positions of circular 'windows'. These 'windows' were filled with a circular piece of wire mesh, which was secured with four cable ties. Each window was 10cm in diameter and located 1cm from the enclosure floor. Measures of the lengths of each part of the enclosure are provided on black arrows.

Each 'encounter group' was placed in the encounter enclosure for 30 minutes. A maximum of one female was placed in each section of the enclosure. All four sections were occupied when high competition encounter groups were tested, but two segments remained empty when low competition encounter groups were tested. Subjects were transferred from their cages to the encounter enclosure using a handling tube (a Perspex tube ~10cm wide diameter with mesh at one end to prevent escape). The handling tube was cleaned with 70% ethanol between transfers. The order in which subjects within an encounter group were placed in the encounter enclosure and the segment in which they were placed was balanced between

treatment blocks, as was the order in which encounter groups were placed in the enclosure. The encounter enclosure was cleaned thoroughly with 70% ethanol between groups.

4.2.4.2.3. Exposure to males: odours and mating

Females were provided with a scent sample from an unrelated male maintained in a separate room on day 1 of treatment blocks commencing on weeks 1, 3, 5, 9 and 11. Scent samples were collected from male cages as described in Section 4.2.4.2.1. Where applicable, scent samples were placed in female cages after sessions in the encounter enclosure were complete. Scent samples were taken from one of two males. Half of the females in each treatment group received the odour of one male, and the other half were presented with scent samples from the other male.

Males used for mating were housed in a separate room except during the 2 weeks they were housed with females. Males were placed individually in female cages after encounter sessions on day 1 of week 7. Males were removed from cages in the order in which they were placed in cages. After removal, males were taken to the room in which they were initially housed.

4.2.5. Corticosterone assays

I used corticosterone assays to investigate whether the concentration of faecal corticosterone excreted by females differed between treatment groups. Here, I detail how samples were collected, the extraction techniques used and the assay procedure.

4.2.5.1. Sample collection

To obtain faecal samples from subjects, I placed them individually in 'collection cages' for 90 minutes. Collection cages comprised of an MB1 cage which did not contain sediment, nesting material or food/water. I transferred females between home and collection cages using a handling tube that was cleaned with 70% ethanol between individuals. After 90 minutes, I removed any faecal pellets present in collection cages using tweezers that were washed with 70% ethanol between individuals. In line with previous studies, faecal pellets in urine were considered 'contaminated' and were not collected [426]. Faecal samples were weighed before being stored at -2^{o}C.

I collected baseline faecal samples from females in the week before they were included in tests. I collected faecal samples from females on two occasions in the treatment blocks commencing on weeks 1, 3 and 5. One sample in each of these treatment blocks was taken

to establish whether faecal corticosterone varied between treatment groups overall. Animals were left undisturbed before this sample was collected. Collection of such samples (termed 'general samples') was completed on day 14 of each of the first 3 treatment blocks. Another sample was taken on the day in each of those treatment blocks that subjects received a scent sample from the conspecific in the same enclosure (Section 4.2.4.2.1). These samples (referred to as 'test samples') were collected to deduce whether the receipt of a cue from a conspecific caused levels of faecal corticosterone to differ between treatment groups. In total, I attempted to obtain three of each 'general' and 'test' samples from each subject alongside a baseline sample.

In bank voles, as in other rodent species, the concentration of faecal corticosterone peaks four hours after a stressful event (e.g. aggressive interactions) [427]. Subjects were undisturbed before 'general' samples were collected, but the change of light could have influenced corticosterone levels. General samples were thus collected 5 hours after the beginning of the dark period to avoid confounding results. Subjects were disturbed when 'test' samples were collected, because scent samples were placed in their cages. I began transferring scent samples between cages approximately 30 minutes after the onset of the dark period. I recorded the time at which each female received an odour and began sample collection 4 hours after that point.

4.2.5.2. Extraction of faecal corticosterone

Extraction was completed for all faecal samples that weighed over 0.01g before being frozen. Samples weighing less than 0.01g were deemed too small to enable reliable measures of faecal corticosterone.

I first ground thawed samples into a fine powder before adding 3ml 90% methanol to each sample. Each sample was then vortexed and agitated overnight (~15 hours). I centrifuged each vial (20 minutes at 1800rpm) and poured the supernatant from each vial in to separate glass test tubes. The supernatants were dried under air in a water bath at ~60^{O}C until the liquid methanol had evaporated (typically after 30-60 minutes). Each extract was reconstituted in 500µl of 100% methanol before being vortexed and then agitated for 15 minutes. Extracts were stored at -2^{O}C.

4.2.5.3. Assay procedure

The concentration of corticosterone in each faecal sample was determined using an enzyme immune assay originally developed by C. Murano (Department of Population Health and

Reproduction, University of California) [428]. The assay was validated in the lab used for tests by myself and Amanda J. Davidson (Mammalian Behaviour and Evolution Group, University of Liverpool, UK).

The assay was conducted using 96-well plates (NUNC-Immuno F96 MicroWell™ MaxiSorp™ ThermoFisher Scientific, Finland). Wells were coated with 50µl of antibody solution (Corticosterone (CC) antibody (CJM006, University of California, California) diluted by a factor of 1:11,000 with sodium bicarbonate solution) before refrigeration (minimum refrigeration before use ~12 hours). Each plate was washed five times using a well washer (Wellwash type 888, ThermoScientific, Finland) before use in assays. I generated standards for the assay by serially diluting a stock solution of 1mg/100ml EtOH corticosterone using EIA buffer (phosphate buffered solution (PBS) containing 0.1% (BSA), pH 7.0). The standard concentrations used were 1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9 and 0pg corticosterone per well. Faecal extract samples and controls were also diluted using the EIA buffer. Two controls (C1 and C2) were created using the corticosterone stock solution. A biological control (C3) was produced using a stock solution which was created by pooling small amounts of each faecal extract produced during the study. Each of the controls were diluted using the EIA buffer. The dilutions used for C1 and C2 were 1:6 and 1:64, respectively. The dilution used for C3 was 1:15. C1 and C2 were created to bind ~30% and ~70%, respectively, whilst C3 bound at ~50%.

Each well of a plate was filled with 50µl of buffer (for blank cells containing no corticosterone), standard, sample or control solutions. I then immediately added 50µl CC horseradish peroxidase (HRP conjugate (University of California), diluted to 1:65,000 using EIA assay buffer) to each well. The plates were then covered and left to incubate in the dark for 2 hours.

Plates were washed 5 times in the well washer after incubation. After washing, I added 100µl room temperature substrate solution (0.5M H_2O_2, 40mM ABTS, 0.05mM citric acid) to each well. Plates were then covered and allowed to incubate in the dark for 30 minutes. After this initial incubation, plates were read at 405nm in a Multiskan FC microplate photometer (ThermoScientific, Finland) every 15 minutes until blank wells (those in which only buffer was added) reached ~0.8 optical density. I attempted to dilute samples from animals such that they reached 0.45-0.55(±0.01) optical density when blank wells reached ~0.8 optical density. If the optical density of samples was outside of this range, then the dilution for that sample was adjusted and this new dilution was tested in a separate assay. Standard curves were

created using Skanit v3.1 software (ThermoScientific, Finland). This information was used to calculate the concentration of corticosterone in each faecal sample obtained from females.

Individual assays were considered to have failed if the coefficient of variation (CV) was greater than 15% between duplicates of a standard or control value on the same plate. Assays were also considered to have failed if there was a significant deviation from a sigmoidal shape in the standardised curve. The results for samples were only accepted if the CV between duplicates of that sample was below 15%. For the results of assays to be reliable, it is important that the measurements recorded from different plates are comparable. The results of different plates can only be considered to be comparable if the inter-plate CV of standards and controls is below 15%.

4.2.6. Measuring traits to investigate maternal effects in offspring

4.2.6.1. Offspring produced in study

Bank vole females are pregnant for approximately 19 days [429]. Males were placed in cages on day 1 of week 7, so the first possible due date of females was day 5 on week 10. Each cage was checked for offspring daily at the beginning of the dark period for 14 days from the first estimated due date. Thus, the age of the pups was accurate to within one day. Pups were usually first detected 20 days after males were initially placed in cages. However, parturition occurred as late as one week later (day 6, week 11). The maximum age difference between offspring produced was seven days in the first study block and one day in the second study block.

In total, 16 litters were produced during the study; 6 in the first study block and 10 in the second. In the first study block most litters were produced by low competition females (2 high competition females and 4 low competition females had litters). However, in the second study block, most litters were produced by high competition females (6 high competition females and 4 low competition females gave birth). As the skew of females that reproduced varied between treatment groups, the treatment was not thought to differentially affect reproductive success of females in different groups. One of the litters produced by a low competition female in the second study block, which contained 2 pups, was lost after 7 days. This litter was not included in any analyses.

Offspring produced during the study were housed in MB1 cages with mothers for 28 days. Housing conditions remained unchanged whilst offspring remained with mothers, except that a nest box (hamster igloo (approximately semi-circular, maximum measurements 14 x

11 x 13cm), Savic, Belgium) was provided when offspring reached 7 days of age. All offspring and respective mothers were pit-tagged (RFID tags, Francis Scientific Instruments, UK) when pups reached 21 days of age by Miss Rachel Spencer (Mammalian Behaviour and Evolution Group, University of Liverpool, UK) with my assistance.

I weaned male offspring singly into M3 cages at 28 days of age. All male offspring were then maintained in the same room as adult males used in the study. In total, 11 males (2 high competition, 9 low competition) were born in the first study block and 18 (11 high competition, 7 low competition) were produced in the second study block. Diabetes tests were conducted when males were ~10 weeks old. In the first block, one male in the low competition group developed diabetes, and two brothers from a high competition litter developed diabetes in the second block.

Adult females that produced only males were retained in MB1 cages until the end of the study block (i.e. when all pups produced in that study block were weaned). Adult females that produced female offspring were transferred to an external enclosure with their female offspring for a separate study (see Chapter 5 for details).

4.2.6.2. Measurements of traits related to body size

To investigate maternal effects on offspring body size, I measured litter size, average offspring growth rate in each litter and offspring weaning weight.

The number of offspring in each litter (i.e. litter size) was first recorded one week after birth, and was monitored weekly until weaning. Besides the litter that was lost after 7 days, no pups are thought to have died, so litter size remained unchanged.

Pups were not individually marked before being tagged, due to increased risk of pup mortality, so growth rate for individual offspring could not be established. Instead, I weighed each litter when offspring were estimated to be 7, 14, 21 and 28 days of age. I then calculated the average growth rate of pups in each litter to investigate whether there was a likely effect of treatment. I calculated the average growth rate of pups in a litter using the formula specified in Vinogradov *et al*. [430]:

$$\text{Growth rate} = \frac{\ln(\text{initial body weight}) - \ln(\text{final body weight})}{\text{Initial time point} - \text{final time point}}$$

The initial body weight here was taken as the average body mass of pups in a litter at day 7, and the final body weight was taken as that at day 28. I calculated the average body mass

of pups in the litter at each of those time points by taking the total weight of the litter and dividing it by litter size.

Pups were pit-tagged before weaning, so weaning weights of individuals were recorded.

4.2.6.3. Measurements of male reproductive traits

Reproductive competition in males may occur before or after copulation [43,53,73,103,107,109,115,120,431,432]. Investment in different competitive strategies is evident in different traits. For example, investment in pre-copulatory competition is associated with increases in body size, whilst investment in post-copulatory competition is associated with increased ejaculate investment [43,53,73,103,107,109,115,120,431,432].

Male bank voles reach sexual maturity at approximately 8 weeks of age [429]. Males were culled at 11 weeks to ensure that subjects were sexually mature when measurements of traits related to reproductive competition were taken. Humane killing was carried out by trained animal technicians (Mammalian Behaviour and Evolution Group, University of Liverpool, UK), using an approved Schedule 1 method with cervical dislocation.

I dissected all males within 2 hours of death. Males were not frozen or refrigerated prior to dissection. All bodies were held in labelled re-sealable bags until dissection. The body mass of males was recorded before dissection. I used diagrams from Rowlands [433] to aid with the identification of reproductive organs. During dissection I removed the epididymides (each epididymis comprised of the cauda- and caput- epididymis joined together [433]), testes and seminal vesicles from each subject. Each organ was weighed in a plastic weighing boat immediately upon removal. The right testicle of each male was stored at -2^{o}C to enable further study on daily sperm production rate.

Estimation of daily sperm production rate was completed by Olivia Antony. The protocol used is identical to that outlined in Ramm and Stockley [424], which was adapted from that in Seung *et al*. [434]. Briefly, testes were thawed for 1 minute before the tunica albuginea was removed, all remaining tissue was then weighed. This tissue was then homogenised in 10ml of dimethyl-sulphoxide/saline solution using a Ystral X10/20 homogeniser with a 10T shaft (Yastral, Germany). Homogenisation occurred in two stages, each lasting 60 seconds. Spermatids were stained with Trypan blue, and spermatid heads were counted under 40x magnification using a Neubauer haemocytometer (Neubauer, USA).

4.2.6.4. Measurements of litter sex ratio

The sex of all offspring was initially determined at 21 days of age when they were pit-tagged. The sexes were confirmed at 23 days of age, when the functionality of tags was checked. This data was used to determine the sex ratio of each litter. One litter was lost before the sexes of pups could be recorded (see Section 4.2.6.1). As no other pups were known to have died during the study, the sex ratio calculated for each litter after 21 days was considered to be the same as the sex ratio at birth. As per convention, sex ratio was denoted as the proportion of offspring in the litter that were male. Thus, litter sex ratios above 0.5 indicate a skew towards more males and sex ratios lower than 0.5 indicate a skew towards females.

4.2.7. Statistical analyses

4.2.7.1. Investigating potential differences in corticosterone concentration between groups

I used a Mann-Whitney U test to investigate whether there was a difference in baseline concentrations of faecal corticosterone of adult female bank voles in each treatment group. This was crucial in establishing that any differences between treatment groups were likely to be due to the effect of the treatments provided to females. Baseline samples were available for 28 out of 32 females; the four females for which baseline samples could not be obtained were excluded from all subsequent analyses.

I used Friedman tests to investigate whether the concentrations of faecal corticosterone varied over time in samples taken from adult female bank voles in each treatment group. Subjects experienced more disturbance prior to the collection of 'test' samples than before the collection of 'general' samples. This could have influenced the levels of faecal corticosterone in different types of sample, so data from different sample types were analysed separately. Specifically, I completed one analysis for females that provided faecal samples during collection periods for baseline samples and 'test' samples, and a separate analysis for those which provided baseline samples and three 'general' samples (i.e. the maximum number of general samples).

I also investigated whether there was a difference in faecal corticosterone concentration between treatment groups. I expected that any effect of treatment on corticosterone would be most evident in samples that were collected later in the study period. I calculated the change in faecal corticosterone concentration between baseline levels and the final 'general' and 'test' samples. I used Mann-Whitney U tests to examine whether there was a significant difference in the level of change evident in females in different treatment groups. As above,

data from ‘test’ and ‘general’ samples were analysed separately, so one test was conducted for each sample type.

4.2.7.2. Statistical analyses to investigate potential maternal effects in offspring

4.2.7.2.1. Linear mixed effects models

I used linear mixed effects models to assess whether there was any evidence of maternal effects due to the treatment applied to adult females. In each model, I included the study block in which the animals were produced as a random effect. In analyses considering individuals rather than whole litters (i.e. male reproductive traits), I also included ‘litter’ as a random effect to account for the relatedness of offspring. Treatment group was included as a fixed effect in all models, but other factors included as fixed effects varied according to the trait under investigation. Previous studies have shown that maternal body mass can influence litter size, birth weight and growth rate [407]. Thus, I included body mass of the mother in analyses of maternal effects on offspring body size (i.e. litter size, growth rate and weaning weight). I additionally included litter size and sex ratio as fixed effects in models of offspring growth rate, as these factors can influence offspring growth rate [63,271,406,410]. As all of the mothers were of a similar age (within 14 days), I did not include maternal age in growth rate models. Litter size was included as a fixed effect in models of weaning weight in accordance with the findings of previous studies [268,270]. Litter size was also included in models investigating differences in male body mass after sexual maturity. Relative ejaculate investment is typically measured as the mass of reproductive organs compared to body mass [102,107,115,120,383]. I thus included body mass as a fixed effect in models assessing the effect of treatment on the size of male reproductive organs. I also accounted for testes mass in models of daily sperm production rate.

To investigate whether there was any effect of treatment on particular traits, I compared a ‘full’ model (i.e. one containing all random and fixed effects) to a ‘test’ model (i.e. one which contained all random and fixed effects besides treatment) using a likelihood ratio test. Where appropriate, values were log-transformed to improve normality.

I found that some males were diabetic at the time of dissection. Observations during dissection indicated that diabetes could influence the size of reproductive organs, particularly the epididymides. Thus, I conducted statistical tests to investigate the effect of treatment on reproductive traits using data from all males and from only healthy males (i.e. those without symptoms of diabetes).

4.2.7.2.2. Statistical analysis of sex ratio data

Sex ratio data are non-normal by definition. I thus used non-parametric analyses for tests. I used Mann-Whitney U tests to assess whether litter sex ratio varied between treatment groups. I then used binomial tests to investigate whether sex ratio differs significantly from equality (i.e. a sex ratio of 0.5) in either group.

4.3. Results

4.3.1. Faecal corticosterone concentration in adult female bank voles

There was no difference in baseline levels of faecal corticosterone between treatment groups (n=28, W=69, P=0.194).

I found no evidence of a significant increase/decrease in faecal corticosterone concentration over time in 'test' samples in either low competition (χ^2=7.40, df=3, P=0.060) or high competition (χ^2=0.43, df=3, P=0.934) adult female bank voles. The near-significant difference in the low competition females appears to be largely due to a relative decrease in the levels of faecal corticosterone by one female in the last test sample. Thus, there is seemingly a near-significant reduction in corticosterone over time in the low competition group. There was no significant increase/decrease in faecal corticosterone concentration over time in 'general' samples in either low competition (χ^2=0.27, df=3, P=0.966) or high competition (χ^2=0.73, df=3, P=0.865) subjects.

I found a near significant difference between treatment groups in the level of change between faecal corticosterone concentration in baseline and the final 'test' samples (n=20, W=75, P=0.056). The level of change was greater in low competition females. There was no significant effect of treatment group on the difference in faecal corticosterone concentration between baseline samples and final 'general' samples (n=25, W=96, P=0.348).

There is evidence that the results of my corticosterone assays may not be reliable. The inter-plate CV of all standards was below 15%, suggesting a high level of consistency between plates. By contrast, the inter-plate CV of controls was more variable (all values ~30%). This suggests that the concentrations of corticosterone in samples may not be comparable between plates.

4.3.2. Maternal effects on traits related to offspring body mass

I measured litter size, offspring growth rate and offspring weaning weight to investigate potential maternal effects on offspring body size. I expected that females in the high

competition treatment group would produce larger offspring if maternal effects were adaptive.

I found no difference in litter size between treatment groups (average litter size (±se): high competition treatment group = 4.38 (±0.57), low competition treatment group = 3.28 (±0.42); χ^2= 1.747, df= 1, P= 0.186).

The average weights of offspring in litters produced by high and low competition females each week until weaning are shown in Figure 4.3. The data in Figure 4.3 indicate that there is little difference in the average offspring body mass either within or between treatment groups at 7, 14, 21 or 28 days of age. There is also little indication that the average growth rates of pups, both between particular weeks and overall, differed between treatment groups (evident from a comparison of slopes in Figure 4.3). Accordingly, I found no difference in the average overall growth rates of pups in litters produced by high and low competition females (χ^2= 0.313, df= 1, P= 0.576).

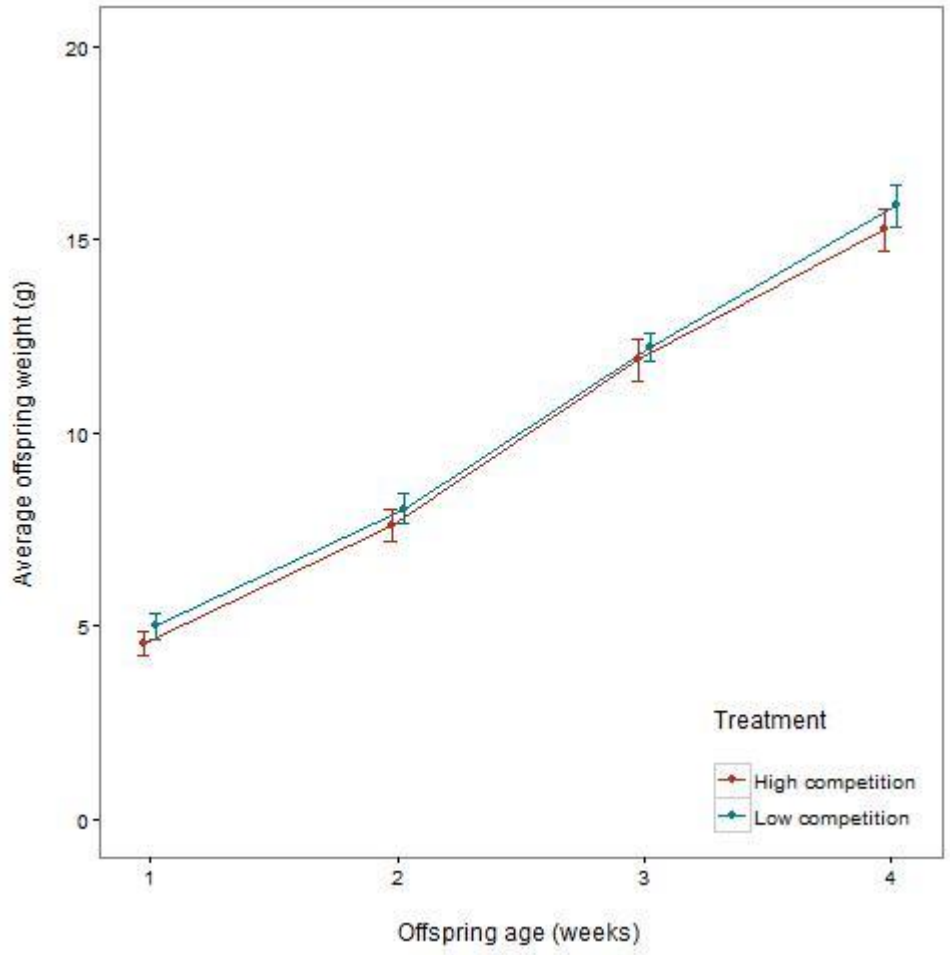

Figure 4.3. Average weights of pups in litters produced by high and low competition females each week from 7 to 28 days of age. See main text for further details on the treatments used in this study. The average weight of pups was calculated by dividing the weight of the litter by the number of pups in each litter. Average values are given ± standard error. Data points on the graph are slightly offset to ensure that the average values and standard error values were clear.

There was no evidence of an effect of treatment group on the weaning weight of male offspring (average weaning weight (±se): high competition = 15.47(±0.46)g, low competition= 16.07(±0.57)g; χ^2= 0.849, df= 1, P= 0.357). Similarly, weaning weight of female offspring did not differ between treatment groups (average weaning weight (±se): high competition = 14.64(±0.39)g, low competition = 14.67(±1.07)g; χ^2= 0.060, df= 1, P= 0.806). This is consistent with the earlier findings that neither litter size nor growth rate differed between groups.

4.3.3. Maternal effects on traits related to reproduction in male offspring

Male bank voles may encounter competition to secure breeding territories and sperm competition [23,293,294,311,394]. As such, I examined maternal effects on traits related to overt contests between males (i.e. body size after sexual maturity) and post-copulatory competition (i.e. size of reproductive organs and sperm production).

4.3.3.1. Maternal effects on male body mass after sexual maturity

I found no evidence of a difference in male body mass between treatment groups. The result was not influenced by whether analyses were conducted using data from all males (average body mass (±se): high competition treatment group = 20.29(±0.39)g, low competition = 19.74(±0.34)g; χ^2= 0.425, df= 1, P= 0.515) or only healthy males (average body mass (±se): high competition treatment group = 20.44(±0.45)g, low competition 19.65(±0.35)g; χ^2= 0.803, df= 1, P= 0.370).

4.3.3.2. Maternal effects on investment in ejaculates

I investigated potential maternal effects on ejaculate investment by assessing whether the relative masses of male sexual organs (specifically testes, seminal vesicles and epididymides) compared to body size and/or daily rate of sperm production differed between treatment groups.

4.3.3.2.1. Testes mass

After controlling for body mass, I found no effect of treatment group on combined testes mass, regardless of whether analyses were completed using data from all males (average testes mass (±se): high competition= 0.52(±0.01)g, low competition 0.52(±0.01)g; χ^2= 0.139, df= 1, P= 0.709) or only healthy males (average body mass (±se): high competition = 0.52(±0.01)g, low competition 0.52(±0.01)g; χ^2= 0.649, df= 1, P= 0.421).

4.3.3.2.2. Seminal vesicles

There was no effect of treatment on the combined mass of seminal vesicles in tests completed using data from all males (average combined mass of seminal vesicles (±se); high competition= 0.16(±0.01)g, low competition 0.14(±0.01)g; χ^2= 0.943, df= 1, P= 0.332) or just healthy males (average combined mass of seminal vesicles (±se): high competition= 0.16(±0.02)g, low competition 0.14(±0.01)g; χ^2= 0.972, df= 1, P= 0.324).

4.3.3.2.3. Epididymides

There was no difference in the combined masses of epididymides between treatment groups when data from all males was included in the analysis (average combined mass of epididymides (±se): high competition= 0.59(±0.06)g, low competition 0.53(±0.03)g; χ^2= 0.008, df= 1, P= 0.928). However, when only data from healthy males were considered, it was evident that combined epididmides mass was significantly higher in males born in the high competition treatment group (average combined mass of epididymides (±se): high competition= 0.66(±0.03)g, low competition 0.54(±0.03)g; χ^2= 4.119, df= 1, P= 0.042) (Figure 4.4).

Diabetic males had smaller epididymides than other males. Thus, the average combined mass of epididymides was relatively higher when data from diabetic males were removed. The resultant change, particularly in the average and standard error values for males born in the high competition treatment group, revealed a significant difference between treatment groups (Figure 4.4.).

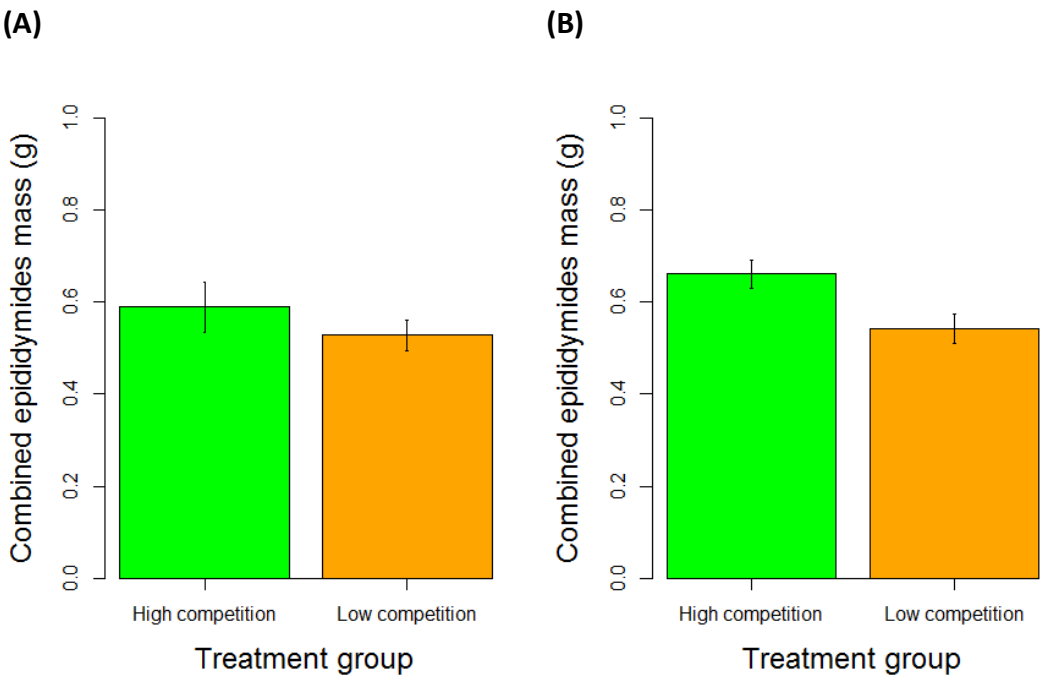

Figure 4.4. Average combined mass of epididymides of male born in high and low competition groups (A) data from all males (B) data from healthy males. Males were born in either the high or low competition treatment group. See main text for details on high and low competition treatments. Data from all males includes data from both healthy and diabetic males. Healthy males consist of males that exhibit no symptoms of any disease, including diabetes. Average combined epididymides mass values are presented ± standard error.

4.3.3.2.4. Rate of daily sperm production

There was no evidence of a significant difference in the daily rate of sperm production in males born in each treatment group when analyses were completed using data from all males (average number of sperm produced per day (±se): high competition= $6.93x10^6$ ($\pm 3.3 x10^5$), low competition $5.99x10^6$ ($\pm 3.6 x10^5$); χ^2= 3.113, df= 1, P= 0.078). However, when data from diabetic males were excluded, there was evidence that daily sperm production rate was significantly greater in males from the high competition treatment group (average number of sperm produced per day (±se): high competition= $7.11x10^6$ ($\pm 3.6 x10^5$), low competition $5.95x10^6$ ($\pm 3.9 x10^5$); χ^2= 6.24, df= 1, P= 0.013). Data on the daily rate of sperm production for all males and for just healthy males are shown in Figure 4.5.

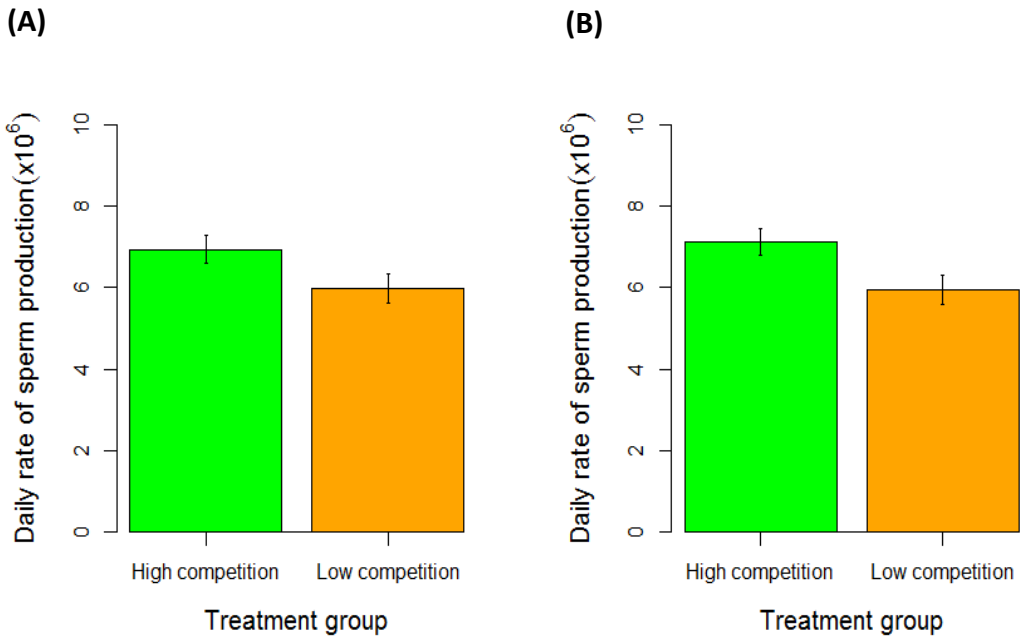

Figure 4.5. Average sperm production per day in high and low competition males (A) data from all males (B) data from healthy males. Males were born in either the high or low competition treatment group. See main text for details on high and low competition treatments. Rate of sperm production indicates the number of sperm produced per day. Data from all males includes data from both healthy and diabetic males. Healthy males consist of males that exhibit no symptoms of any disease, including diabetes. Average sperm production rate values are presented ± standard error.

4.3.4. Maternal effects on litter sex ratio

The sex ratio of litters produced by females in the high and low competition was significantly different between groups (W=8, P=0.022). Sex ratio was relatively female-biased in litters produced by high competition females, and more male-biased in the litters of low competition females (litter sex ratio (±se): high competition = 0.354 (±0.07), low competition = 0.764 (±0.12)) However, binomial tests showed that litter sex ratio did not differ significantly from equality (i.e. 0.5) in either treatment group (high competition: n=35, P=0.176; low competition; n=23, P=0.093). The average sex ratio of litters produced in each treatment group are shown in Figure 4.6.

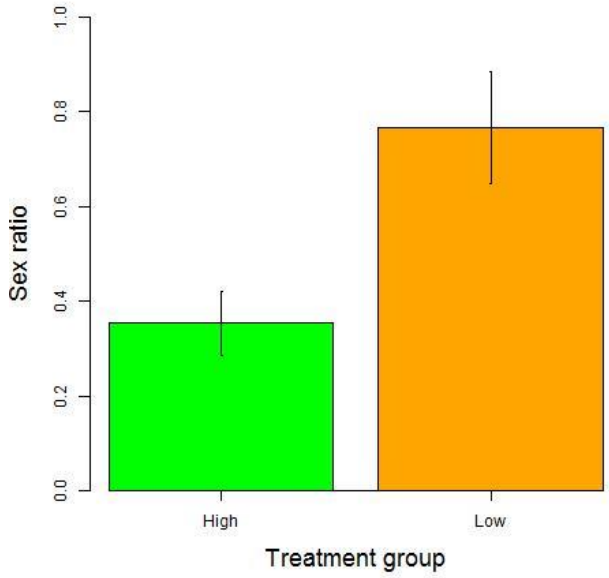

Figure 4.6. Average sex ratio of litters produced by high and low competition females. Treatment group indicates whether females were subject to social cues indicating 'high' and 'low' levels of population density. See main text for details on high and low competition treatments. Sex ratio indicates the sex ratio of litters produced as a proportion of males present in litters. Average litter sex ratio values are presented ± standard error.

4.4. Discussion

4.4.1. Faecal corticosterone concentrations in adult female bank voles

Previous studies have shown that levels of glucocorticoids may be elevated under high density conditions [255,276,387,435]. This is often thought to be related to increased levels of aggression and decreased resource accessibility in densely populated habitats [24,141]. However, there is evidence that indicates that these are not the only factors causing levels of glucocorticoids to be relatively increased in individuals in high density environment. Dantzer *et al*. [387] used vocal cues to simulate high density conditions, and found that levels of glucocorticoids were relatively increased in female *T. hudsonicus* in 'high' density conditions, even though resource accessibility was not manipulated during tests.

Contrary to expectations based on the results of previous studies, I found no evidence of an effect of treatment group on faecal corticosterone concentration. This could indicate that cues of population density alone were insufficient to influence corticosterone levels in female bank voles before pregnancy. Alternatively, it is possible that differences in corticosterone

due to cues of population density are only evident during pregnancy, which is when corticosterone levels were found to differ in previous studies [387,397,398,436]. However, the results should be considered with caution, given that inter-plate CV values for controls suggest that corticosterone concentrations of samples tested on different plates may not be comparable. This means that results based on a comparison of the concentration of faecal corticosterone in samples tested on different plates, as in analyses here, may not be reliable. Ideally, samples would be reanalysed, but many samples were exhausted whilst completing assays.

There were several issues with conducting the corticosterone assay, and these may have contributed to the high inter-plate CV values for controls. The corticosterone assay used is incredibly temperature sensitive [428], with inferred concentrations of faecal corticosterone fluctuating according to the temperature in which the assay was performed. I was unable to conduct assays in temperature-controlled conditions, and temperature in the lab varied between days. The high inter-plate CV for controls could have been caused due to the effects of temperature variations between assays on different days and at different time periods during the day. I attempted to improve the reliability of results by recompleting assays, but I exhausted samples before I was able to significantly improve results.

4.4.2. Maternal effects on competitive ability to acquire resources besides mates

Larger animals typically have an advantage in high density conditions, where levels of social competition are likely to be high, as they are more able to secure access to resources [437]. Maternal effects can influence offspring body mass by acting on at least one trait related to birth weight, litter size and/or growth rate [263,275,387,438]. Maternal effects on body weight should be evident in offspring weaning weight [268,270].

4.4.2.1. Litter size

The Smith-Fretwell model predicts that there should be a trade-off between offspring size and number, with smaller litters typically containing larger offspring [406]. Thus, females in the high competition group could have adaptively influenced offspring body mass by producing relatively small litters.

I found no evidence of a maternal effect on litter size, suggesting that mothers did not alter litter size in order to influence offspring body mass. This finding conflicts with studies which indicate that females may adaptively influence offspring size in accordance with the Smith-Fretwell model [406,410]. However, it is consistent with the results of previous studies that

suggest that resource availability is likely to be the main factor affecting litter size in bank voles [295,307,439]. To date, there is no evidence that female bank voles alter litter size during pregnancy in response to any factor, including population density. Indeed, previous studies have found no evidence of an effect of population density on initial litter size [283]. There is evidence that female bank voles may influence litter size post-partum, as the weaning success of female bank voles is influenced by resource availability and density [295,307,439] It is therefore possible that a maternal effect on litter size would have been evident if resource availability had varied between groups, and that cues of population density were insufficient to cause an effect.

4.4.2.2. Growth rate

Previous studies have shown that maternal effects on offspring growth rate may be adaptive or maladaptive [253,263,267,271,387,403]. Maternal effects on growth rate are usually considered to be maladaptive if they cause delayed sexual maturity and/or relatively small adult body mass, both of which could negatively impact lifetime reproductive success [253,271]. Maladaptive maternal effects typically result where the mothers access to resources is limited [253,271]. By contrast, maternal effects on growth rate are generally thought to be adaptive if they cause relatively large adult size and/or faster sexual maturity [267,387,403]. Dantzer *et al.* [387] showed that social cues indicating high density could cause adaptive maternal effects on growth rate in the American red squirrel (*T. hudsonicus*). Thus, I expected that female bank voles subject to cues indicating high density would produce faster growing offspring.

Contrary to initial predictions, I found no evidence of any maternal effect on offspring growth rate in bank voles. This indicates that social cues are insufficient to cause a maternal effect on growth rate in bank voles, inconsistent with the results of Dantzer *et al*. [387] on *T. hudsonicus*. To my knowledge, no other studies have considered maternal effects on growth rate due to cues indicating different levels of social density. However, several studies on maternal effects on growth rate indicate that resource availability may be relatively more important than the number of other individuals present *per se*. For example, in wild savannah baboons (*Papio cynocephalus*) the growth rate of offspring is related to the dominance status of mothers [271]. Females with a higher social rank (i.e. those that are more dominant) have greater access to resources than lower ranking females [271]. As a result, females in lower social ranks produce offspring that grow relatively slowly [271]. Similarly, in harbour seals (*Phoca vitulina*) heavier females, which were likely to be able to provide more milk to young,

produced faster growing offspring that matured faster and had greater body mass at weaning [267]. The finding that there was no difference in offspring growth rate between treatment groups in the present study may have resulted because resource availability was the same in each group. As such, the result could be considered consistent with studies assessing the effect of resource availability on offspring growth rate.

4.4.2.3. Weaning weight

Contrary to initial expectations, I found no evidence of a maternal effect due to treatment group on offspring weaning weight.

The absence of a maternal effect on weaning weight in bank voles is consistent with the results of a similar study by Marchlewska *et al*. [310]. In that study, higher rates of competition and population density were simulated by allowing short but frequent encounters with same-sex conspecifics during three days in late pregnancy. The weaning weight of offspring produced by females exposed to same-sex conspecifics was not significantly different from the weaning weight of offspring born in the control group. Although the results of the study by Marchlewska *et al*. [310] are similar to that those in the present study, there were marked differences in the methodologies used. Alongside the difference in treatments applied to females, pups were also weaned at a younger age in the earlier study (day 20, rather than day 28). Thus, the weaning weights of pups in the two studies are not directly comparable. A more direct comparison could be obtained by considering data on average pup weight on day 21 collected in this study (shown in Figure 4.3). Although the data considers average pup weight rather than the weights of individual offspring, it is clear that offspring body weight is similar between treatment groups at that age. The fact that the results are similar despite the use of different methodologies indicates that results are likely to be reliable. The results of the two studies taken together indicate that social cues simulating different levels of population density and inter-female competition are unlikely to cause maternal effects on offspring body size in bank voles.

The absence of a maternal effect on weaning weight is somewhat consistent with results on previous studies, but only when considered together with the results related to litter size and offspring growth rate (Sections 4.4.2.1-2). Maternal effects on weaning weight are often considered alongside those on other traits related to offspring body size, such as offspring growth rate. For example, in the study on *P. vitulina* mentioned above, heavier females produced faster growing pups [267]. This relative increase in growth rate caused the weaning weights of offspring of heavier females to be relatively high. If there is no maternal effect on

traits associated with offspring growth rate, then a difference in weaning weight would not be expected. I did not examine all traits associated with offspring growth rate, but the result is consistent with findings on offspring growth rate and litter size in this study. The fact that weaning weight does not differ between treatment groups in the present study could indicate that that traits related to offspring body size which were not recorded in this study, including birth weight, may also be unlikely to differ between treatment groups.

4.4.3. Maternal effects on reproductive traits in males

Bank vole males, as with males in most mammalian species, do not provide parental care and are thus more likely to compete for mates than for access to resources associated with successfully rearing young [12,24,141,144]. When competition for mates is high, as in densely populated habitats, males may increase investment in traits that may confer an advantage in reproductive competition [60,109,115,124,126]. For example, males may increase investment in ejaculates if the risk of sperm competition is high [60,103,115,117,120], alternatively males may invest in traits associated with contest competition, including armaments and larger body size [43,96,109,124,126].

Bank vole males are territorial during the breeding season [23,293,294,311,394], and larger size may confer an advantage in gaining territories. Thus, I expected that body size may be larger in high density populations where competition for territories will be high. Similarly, I expected that the risk of encountering sperm competition would be greater in high density populations [81,107], given that bank voles are promiscuous [23,293,294,311,394]. I therefore predicted that adaptive maternal effects would cause investment in ejaculates to be greater in sexually mature males born in the high competition treatment group.

4.4.3.1. Male body mass

Contrary to expectations based on the possible adaptive benefits of larger body size in gaining territories, the body mass of sexually mature males did not differ between treatment groups. The absence of a difference may indicate that larger body mass at sexual maturity does not confer a significant advantage to male bank voles. Although male bank voles do exhibit territoriality during the breeding season, there is often overlap between male territories [304,313,316]. This is an indication that males are not strictly territorial. Therefore, males may not experience high levels of aggression from same sex conspecifics, even in the breeding season. If this is the case, then larger body size may not comprise a significant advantage in securing and maintaining access to breeding territories. This is consistent with the fact that bank voles do not exhibit sexual size dimorphism. Conversely, investment in

ejaculates is likely to improve reproductive success in the bank vole, particularly where the risk of sperm competition is relatively high. Several studies have found evidence of a trade-off between investment in pre-and post-copulatory competition [109,124,126]. Thus, bank vole males may preferentially invest in ejaculates, rather than in traits associated with pre-copulatory competition.

The fact that male body mass after sexual maturity does not differ between treatment groups is consistent with previous results in this study related to offspring growth and size before sexual maturity (Section 4.4.2.). Maternal effects which cause increased offspring body size at weaning typically result in offspring maturing at an earlier age [387] and/or having a larger adult body mass [253]. Indeed, differences in body size at weaning often persist throughout the lives of individuals [253]. For example, in the white tailed deer (*Odocoileus virginianus*), offspring raised in environments where resource availability is low are significantly smaller than those raised in good quality habitat, both at weaning and in later life [253]. The fact that I found no difference in traits related to offspring body mass at weaning is thus consistent with the finding that maternal effects do not influence male body mass at sexual maturity.

4.4.3.2. Traits associated with ejaculate investment

4.4.3.2.1. Testes mass

After controlling for body mass, I found no effect of the treatment on testes mass in male bank voles. This was contrary to both expectation and the results of a previous study examining maternal effects in bank voles [310]. Marchlewska-Koj *et al.* [310] found that females exposed to same-sex conspecifics during pregnancy produced male offspring with significantly smaller testes than those born to control females. This indicated that high population density caused reproductive suppression in male offspring, in line with the results of studies on other vole species [255,260]. It is possible that the disparity between the results of this study and that by Marchlewska-Koj *et al.* [310] is due to the difference in age at which testes were measured. Marchlewska-Koj *et al.* [310] measured testes mass at weaning (20 days old), whilst I measured testes mass when males reached 11 weeks (77 days old). A delay in sexual maturation due to high population density and/or competition between conspecifics, like that seen in Marchlewska-Koj *et al.* [310], may only have been evident in earlier stages, which were not recorded in the present study. If this were the case, it would indicate that high population density caused delayed sexual maturity in males, and not complete reproductive suppression.

The absence of a difference in testes mass between groups is, however, consistent with a previous study examining how male bank voles adjust ejaculate investment in relation to the risk of sperm competition. Lemaître *et al*. [102] presented social cues to adult male bank voles to simulate a 'high' and a 'low' risk of sperm competition, using a method similar to that in the present study. Both theoretical studies and experimental studies on other species suggested that an increase in sperm production would be advantageous when males were more likely to experience sperm competition [102,107,357,424]. However, Lemaître *et al*. [102] found no effect of treatment on testes mass, indicating that male bank voles do not alter sperm production in response to social cues of sperm competition. Instead, the authors suggested that other forms of ejaculate investment may be relatively more important for ensuring reproductive success in male bank voles when the risk of sperm competition was high.

4.4.3.2.2. Seminal vesicle mass

I found no evidence of a maternal effect on seminal vesicle mass relative to body mass. To my knowledge, this is the first study to examine whether maternal effects influence seminal vesicle mass. The components of ejaculates produced in the seminal vesicles are important for the production of copulatory plugs [102]. Seminal vesicle mass is thus expected to be increased where copulatory plugs are relatively important in reproductive success. Given that previous studies on vole species indicate that high density is associated with reproductive suppression in males, males born in the high competition group were expected to have decreased investment in reproductive traits. Such an effect is not evident on seminal vesicle mass, indicating that investment in copulatory plugs is not subject to a maternal effect due to the social cues provided to adult females. By extension, the results suggest that the level of social competition under different population densities does not cause a maternal effect on investment in copulatory plugs.

The finding that there is no effect of likely risk of sperm competition on seminal vesicle mass is inconsistent with the results of Lemaître *et al*. [102], which suggested that copulatory plugs were likely to be relatively important when the risk of sperm competition is high. Specifically, Lemaître *et al*. [102] found that males subject to cues indicating a high risk of sperm competition had relatively large seminal vesicles. One of the key differences between that study and the present study is that here I evaluated maternal effects, rather than changes in investment in adult males in response to cues of social competition. It is possible that maternal effects do not influence in seminal vesicle mass, but that sexually mature males are

able to alter investment in copulatory plugs in response to the risk of sperm competition in their environment.

4.4.3.2.3. Mass of epididymides and daily rate of sperm production

Contrary to initial expectations based on reproductive suppression under high density conditions, I found evidence of adaptive maternal effects on both the combined mass of epididymides and the daily rate of sperm production. Specifically, males born in the high competition group had significantly larger epididymides and a higher rate of daily sperm production than males born in the low competition group. Both results can be considered evidence of adaptive maternal effects on male reproductive traits, as it indicates that ejaculate investment in greater when the risk of competition is relatively high. Neither epididymides mass or daily rate of sperm production were explicitly considered in earlier studies on maternal effects in vole species, including bank voles [255,260,310]. However, reproductive suppression was evident in those species when individuals were present in densely populated habitats [255,260,310], or when individuals receive cues indicating increased density/social competition [102,310]. Moreover, in a previous study on bank voles, Lemaître *et al*. [102] found that cues of social competition did not influence sperm production. Thus, the results of the present study are seemingly in opposition with those in previous studies on maternal effects in vole species.

Whilst the findings of investigations on epididymides mass and daily sperm production rate are in conflict with those in earlier vole studies, they are consistent with the results of studies on other species. For example, Ramm and Stockley [424] found that daily sperm production and epididymal sperm counts were elevated under high competition conditions in the house mouse (*Mus musculus domesticus*). That study used method similar to that in the present study to simulate high levels of sperm competition, social cues were applied directly to adult males, rather than their mothers. The results of Ramm and Stockley [424] reflect a seemingly adaptive strategy in response to high levels of mate competition in house mice, in line with the maternal effect on male reproductive traits evident in male bank voles in the present study.

One unexpected finding from this study was the apparent effect of diabetes on reproductive traits, particularly the masses of epididymides and daily sperm production rate. Indeed, maternal effects on these traits were only evident after data from diabetic males were excluded from the analyses (n=3). Diabetic males exhibited symptoms consistent with the Ljungan virus, which is present in wild bank vole populations in the UK [422]. Symptoms of

diabetes became evident shortly before males reached 11 weeks, and all bank voles were tested before dissection. I attempted to eradicate diabetes from the captive population by selectively breeding asymptomatic animals. None of the adult animals included in the study exhibited diabetes (see Sections 4.2.1.), nor did they subsequently develop the disease. Procedures were in place throughout the study to minimise any possible transmission between animal rooms in the animal unit. Whilst I feel that these procedures likely minimised the incidence of diabetes in offspring, they were obviously insufficient to completely prevent the development of diabetes in offspring.

4.4.4. Maternal effects on litter sex ratio

The local resource competition (LRC) hypothesis posits that females should produce lower numbers of the more philopatric sex where competition for resources is high [416]. In bank voles, females are the relatively more philopatric sex, as they are less likely to disperse from the natal area [23,318]. I therefore predicted that females exposed to social cues indicating high density would be more likely to produce litters with a male-biased sex ratio.

I found a significant difference in sex ratio between treatment groups, consistent with initial expectation. However, contrary to the LRC hypothesis, the sex ratios of litters produced by high competition females were more female-biased (Figure 4.6). If maternal effects on litter sex ratio were adaptive, females in the high competition group should produce more males (i.e. the more dispersive sex [23,318]). This would be adaptive as males would disperse away from the natal area, and the level of competition in the area in which the mother was resident would not be increased. Thus, the relative over-production of females by females in the high competition group appears maladaptive.

The relative-over production of females in high density conditions could be explained due to the flexibility of female dispersal behaviour and altruism between related females. Firstly, female bank voles are not strictly philopatric [23,318], and increased densities are thought to cause higher rates of female dispersal [311]. Thus, increased production of female offspring under higher densities would not necessarily cause exponential increases in competition within the territory of an adult female. Secondly, the reproductive success of female bank voles can be higher when they breed in kin groups [311]. Mappes *et al*. [311] found that the home ranges of relatives overlapped more than those of unrelated individuals, and that kin selection may occur. Data from previous studies indicates that infanticide rates may be lower when the home range of a female borders a related, rather than an unrelated, female. It is possible that female bank voles produce more females under high density

conditions in order to benefit from kin selection. Conversely, Mappes *et al*. [311] predicted that higher dispersal rates under high density conditions would cause the breakdown of female kin groups and that levels of altruism between females would be negatively associated with resource competition. In this case, it is unlikely that females can benefit from breeding in kin groups under high density conditions. Further testing would be required in order to better understand why litter sex ratios would be female-biased in high density environments.

Few previous studies have tested the LRC hypothesis in mammalian species, largely due to the logistical complexities of conducting such studies [416]. It is therefore difficult to assess support for this hypothesis relative to others that make predictions about alterations to litter sex ratio, including the Trivers-Willard hypothesis [440], across mammals. A recent experiment in tammar wallabies (*Macropus eugenii*) used cross-fostering to assess evidence for the LRC hypothesis [416]. It found partial support for the hypothesis, as it showed that rearing an additional daughter reduced future reproductive fitness of the mother [416]. Cross-fostering experiments in bank voles may enable a more direct assessment of the LRC in this species.

Despite the significant difference in the sex ratio of litters produced by different females, I found no evidence that the sex ratio differed from equality in either group. This may be due to the low numbers of litters produced in the study. Alternatively, it may reflect that the significant difference in sex ratio detected in this study was not the result of a maternal effect. As such, this result must be considered with caution.

4.5. Conclusions

Although I did not find evidence of an effect of population density on the levels of glucocorticoids expressed by females, problems with the assay procedure mean that I was unable to determine the reliability of this result. Thus, I could not assess whether any maternal effects were likely to be related to concentrations of maternal stress hormones.

I found no indication of a maternal effect on traits related to offspring body size, including male body size at sexual maturity. This could indicate that faster rates of sexual maturation and increased body size do not increase reproductive success where population density, and by extension social competition, is high. Contrary to the results of previous studies, I found no evidence of reproductive suppression in offspring produced under simulated conditions of relatively high density. Instead, maternal effects acted adaptively on some reproductive traits in males. Specifically, the mass of epididymides and daily sperm production rate were

greater in the male offspring of females subject to social cues indicating high population density. The sex ratio of litters differed significantly between treatment groups, but in a manner inconsistent with the local resource competition (LRC) hypothesis. Litters produced in in the high competition treatment group were skewed towards females, whilst those in born in low competition treatment group were male-biased. This appears to be the result of a maladaptive maternal effect on litter sex ratio. However, it is possible that, due to altruism between related females and/or flexible female dispersal behaviour, reproductive success will not be negatively impacted by the relative overproduction of females in high density conditions. Further studies are required to determine both whether the result indicates a maternal effect on sex ratio and whether it is likely to be maladaptive.

Chapter 5: Does social competition experienced by female bank voles (*Myodes glareolus*) influence natal dispersal behaviour of their offspring?

Abstract

High levels of social competition can cause individuals to become excluded from resources required for survival and/or reproduction. Maternal effects can act adaptively on natal dispersal behaviour by causing offspring born in areas where social competition is high to be more likely to disperse and/or to disperse further. I investigated the influence of different levels of social competition on maternal effects on dispersal in the bank vole (*Myodes glareolus*). I presented social cues to adult female bank voles under carefully controlled conditions to simulate 'high' and 'low' levels of social competition in the maternal habitat. The natal dispersal behaviour of offspring of each sex produced by these females was assessed indirectly by evaluating boldness in males under laboratory conditions and movements of females in an outdoor semi-natural enclosure. I found no evidence of a maternal effect on natal dispersal behaviour in either sex. This could indicate that cues of social competition are insufficient to cause maternal effects on dispersal behaviour. Other factors associated with social competition, such as resource availability, may instead be crucial to drive maternal effects on natal dispersal behaviour.

5.1. Introduction

5.1.1. Factors affecting natal dispersal behaviour

Natal dispersal can be defined as a permanent movement of individuals from the site of birth (i.e. the natal area) to the population in which first breeding occurs [24]. The dispersal behaviour of individuals is critically important in population persistence; dispersal influences population dynamics [142,145,156,229], the ability of populations to cope with environmental change [156,195] and the likelihood of inbreeding depression [24,142,156,191,441]. Several studies have attempted to determine the proximate and ultimate causes of dispersal, but such causes remain somewhat obscure [142]. Greenwood [24] identified inbreeding avoidance, local mate competition and local resource competition as ultimate drivers of natal dispersal. More recently, kin competition has also been identified as an ultimate cause [142,196]. Some proximate causes of dispersal include seasonal cues [177,322], parental aggression [442–444], body condition [156], physiological cues [322] and

parental effects (i.e. maternal and paternal effects) [445–447]. In this chapter I focus specifically on potential maternal effects on dispersal.

5.1.2. The impact of maternal effects on natal dispersal propensity

5.1.2.1. Maternal effects on dispersal could be beneficial

Dispersal is a costly behaviour. Perhaps the largest and most obvious energetic cost is that associated with undertaking long-distance movements between groups and/or areas [24]. Another sizeable energetic cost in some taxa is the development of limbs/appendages explicitly for dispersal (e.g. wings in the alder aphid (*Pterocallis alni*) [242]). The costs of dispersal are, however, not exclusively related to energetic expenditure. By dispersing, individuals may delay the time of first breeding [192]. This could result in decreased lifetime reproductive fitness, unless animals are able to compensate by having greater reproductive success after dispersal than would have been possible otherwise [448]. Individuals that disperse are also subject to higher levels of predation risk [56,243,449,450]. Lastly, individuals may experience outbreeding depression if they are relatively poorly adapted for the environment in which they settle [451]. Given the significant costs associated with dispersal, individuals should only be expected to disperse if these costs are outweighed by the reproductive fitness benefits of doing so. An incorrect assessment of the costs and benefits of dispersal could severely negatively influence lifetime reproductive fitness. Thus, although individuals will never have perfect knowledge [250], they must make a reasonable assessment of whether dispersal is likely to be beneficial.

In the case of natal dispersal, juveniles must make dispersal decisions within relatively short periods of time [250,390,398,399]. This time constraint could theoretically decrease the level of accuracy with which juveniles can assess the relative costs and benefits of dispersal. Several studies have shown that mothers may attempt to maximise offspring fitness by influencing offspring phenotype to increase the likelihood of dispersal or philopatry [250]. Manipulations of the phenotype of offspring by the mother that are not related to genotype are termed maternal effects [247,248], and are a form of parental effect. There is evidence that many factors, including resource availability [236,452], population density [250,389], risk of predation [446], maternal stress [399] and parasite load [396,453] may cause maternal effects on dispersal propensity in species across birds [396,452,453], mammals [250] and reptiles [236,399,403,445,446] (summarised in [250]).

5.1.2.2. Are maternal effects on dispersal likely to be adaptive?

Maternal effects are considered adaptive where they improve offspring survival and fitness. However, there has been some debate as to whether maternal effects on any trait are adaptive [404,454,455]. To properly assess whether maternal effects are likely to be adaptive, it is critical that they are considered within the ecological context of the species [454]. Without this context, maternal effects on traits may appear to have a negative effect whilst actually benefitting offspring [454].

There is evidence that maternal effects on natal dispersal can be maladaptive (i.e. maternal effects negatively influence offspring fitness and/or survival). In the cowpea weevil (*Callosobruchus maculatus*) older mothers produce significantly more offspring with wings (otherwise known as the 'active-form') and consequently disperse [456]. Sano-Fujii [456] hypothesised that this maternal effect occurred because older mothers produced less viable eggs which were more susceptible to factors which induce the active-form of the species. Hence the production of relatively more active-form young by older females does not appear to pre-adapt offspring to the maternal environment, and seems unlikely to maximise reproductive fitness. Instead, it may cause offspring to disperse when philopatry would actually be more beneficial.

In most cases though, maternal effects on dispersal behaviour are thought to be adaptive. For example, Bestion *et al*. [446] found that exposure to predator odours during pregnancy caused a significant increase in offspring dispersal propensity in the common lizard (*Zootoca vivipara*). This could be considered an adaptive antipredator response, as young are more likely to survive if they avoid high levels of predation risk.

Maternal effects on dispersal, like maternal effects on other traits, are thought to be regulated by maternal hormones. Exogenous application of the glucocorticoid corticosterone (a stress hormone) to mothers increases the likelihood of natal dispersal in *Z. vivipara* offspring [398]. This result has been used to suggest that stressful conditions such as resource limitation and high social competition in the maternal environment could increase the dispersal propensity of young [251,397–399,403,454,457]. This could be an adaptive response, as it would imply that juveniles avoid conditions that could restrict reproductive success. Other types of hormones besides glucocorticoids have also been associated with maternal effects on dispersal behaviour. In great tits (*Parus major*), higher levels of androgens transferred to eggs by the mother [276,395,453] were associated with greater natal dispersal distances in offspring [396]. Tschirren *et al*. [453] found that lower levels of

androgens were present in eggs when nests were heavily infested with ectoparasites. The relative reduction in natal dispersal distance in response to high levels of ectoparasites in nests is thought to increase the fitness of both the mother and offspring [396,457].

Maternal effects may act directly on dispersal, as indicated in the studies described above, but may also indirectly influence dispersal behaviour by influencing associated traits such as boldness or sociality [231]. The relationship between such traits and dispersal is highly context dependent. Offspring that exhibit a greater degree of sociality may be unlikely to disperse from a high density environment, but may readily disperse from a low density habitat in which they may achieve a greater level of reproductive success [231,458]. In some species, boldness is positively correlated with dispersal behaviour, with bolder individuals more likely to disperse [231]. In others, increased boldness causes reduced dispersal propensity [230], particularly where bolder individuals are more likely to be at risk of mortality [231].

One of the best studied factors causing a maternal effect on natal dispersal behaviour is population density, and thus the level of social competition, experienced by the mother during pregnancy. Such maternal effects will be the primary focus of the present study.

5.1.3. Population density and consequent competition cause maternal effects on dispersal

Associations between population density experienced by the mother and offspring natal dispersal behaviour are increasingly recognised to be highly complex [459]. At high levels of population density there are high levels of social competition, which could negatively impact individual reproductive fitness if it causes a reduction in the accessibility of resources, including mates [389]. Maternal effects can act to help offspring escape the negative effects associated with high levels of population density by promoting dispersal [403].

It is relatively rare for high population density to promote philopatry rather than dispersal [403]. However, there is evidence that females in high density populations may produce relatively philopatric young in the common lizard (*Z. vivipara*) [398,403]. High levels of population density and social competition are associated with an increase in maternal corticosterone in *Z. vivipara* [398,454]. De Fraipont [398] found that high levels of pre-natal maternal corticosterone promote natal philopatry in offspring. The example of the common lizard is particularly interesting as population density during gestation has been shown to be both positively [389] and negatively [398] correlated with dispersal behaviour; suggesting an adaptive response depending on the particular circumstances faced.

Several hypotheses have been proposed to explain why increased population density and social competition could cause a reduction in dispersal behaviour [403]. High levels of social interaction may inhibit dispersal behaviour if the probability of encountering aggression from conspecifics is greater outside of the natal social group. It is also possible that negative associations between pre-natal population density and natal dispersal propensity can be explained by habitat selection theory. Lastly, high numbers of conspecifics in an area may indicate good quality habitat, a reduction in density could signal habitat deterioration, resulting in higher levels of dispersal at lower population densities (discussed in [403]).

Several studies have found that maternal effects act to cause increased offspring natal dispersal propensity and/or distance when population density is high [403]. Such an effect has been observed in several invertebrate species. In the alder aphid (*Pterocallis alni*) the number of winged offspring represents the number of dispersing individuals. A high population density promotes the production of winged offspring [242]. This maternal effect is considered to be a key determinant of population cycles [242], and could be adaptive if it prevents the overexploitation of resources in a given area. More recently, Bitume *et al.* [239] found that higher density in the parental and grand-parental environments caused longer natal dispersal distances in the spider mite, *Tetranychus urticae*. There is also considerable evidence of a positive relationship between prenatal population density and offspring natal dispersal behaviour in vertebrates. For example, Léna *et al*. [389] found that juvenile *Z. vivipara* were more likely to disperse when the density of adult females was high in outdoor enclosures. Moreover, natal dispersal propensity was greater in offspring of the tropical damselfish (*Ponacentrus amboinensis*) produced in high density populations [454]. In each case, dispersal under conditions of high population density could be considered adaptive as it allows individuals to avoid the elevated costs associated with living in a densely populated habitat.

Several factors associated with high population density may drive dispersal behaviour, including restrictions in food availability and high levels of social competition [242,447]. It can therefore be difficult to discern which factors associated with population density drive maternal effects on dispersal behaviour [242]. However, some studies have attempted to observe factors associated with high population density in isolation. Massot and Clobert [447] investigated the potential impact of resource availability. They found that female *Z. vivipara* that were well provisioned produced offspring that were more likely to disperse. Mashanova *et al*. [242] instead investigated how the numbers of individuals may impact dispersal behaviour independently of food quality and availability. The model created was

based on *P. alni*, an aphid for which the level of resources remains reasonably constant throughout the breeding season. The model predicted that high numbers of individuals was associated with an increase in dispersal propensity independently of resource availability. In this study, I specifically consider how the number of individuals in the maternal environment (independent of resource availability) influences natal dispersal behaviour of male and female offspring in the bank vole (*Myodes glareolus*).

5.1.4. Natal dispersal behaviour in the bank vole (Myodes glareolus)

The bank vole (*Myodes glareolus*) is an arvicoline rodent that adopts a generalist diet and is a common resident across the Palaearctic zone [283–285]. Both male and female bank voles are territorial during the breeding season. Inter-sexual territorial overlap facilitates a promiscuous mating system [23,293,311,394]. Members of both sexes may disperse from the natal area, and can travel distances of up to ~1km [316,460]. Natal dispersal in bank voles is generally male-biased, as females typically disperse less frequently and over shorter distances [294,295,316,320,461]. However, individuals of both sexes may disperse [23,318]. Natal dispersal behaviour of individuals of both sexes can be impacted by several factors, including population density and season [23,311,319,462].

Female bank voles can produce up to 3 litters in a year, which are often multiply-sired [23,316]. The first litter of the year is produced in spring, and offspring from this first litter are more likely to disperse than offspring in later litters [23,294,460]. Adult females often share territories with female offspring from later litters throughout the year [23,311]. Female offspring from the first litter of the year are more likely to reach sexual maturity and reproduce in the year of birth than those from later litters [23]. There is evidence that the dispersal of the first litter is beneficial to both dispersers and resident adult females, as it lowers local resource competition and maximises fitness for all individuals [23]. Individuals are most vulnerable to mortality whilst overwintering [296]. Lifetime reproductive success may therefore be greatest in individuals born in the first litters of the year, although Tkadelc and Zejda [463] found that females rarely breed in two consecutive years. As well as the timing of birth, White *et al.* [319] found that bank vole dispersal is also influenced by the interaction of habitat suitability and population density. In line with density-dependent dispersal behaviour [139], dispersal in bank voles occurs more frequently where population density is high [311,319]. Dispersal is also expected to occur more often where habitat suitability is high [319]. Models in White *et al.* [319] indicate that the interaction between population density and habitat suitability influences the threshold at which density induced

dispersal, the number of individuals dispersing and the direction in which individuals disperse.

5.1.5. Aims of this study

To my knowledge, no previous study has considered the influence of social competition in the maternal environment on offspring natal dispersal in bank voles. However, there is evidence that certain traits in bank voles are influenced by maternal effects (see Chapter 4). Here I predict that the social environment of bank vole mothers will impact the dispersal propensity of their offspring, as has been observed across several taxa (see Section 5.1.3.). I expect that the offspring of mothers that have been exposed to cues indicating high levels of social competition will be more likely to disperse. Using controlled experiments, I investigate how cues of social competition presented to adult females influence the natal dispersal propensity of their offspring. As natal dispersal occurs more frequently in male juveniles [316,320], I anticipate that a maternal effect may be more evident in females.

5.2. Methods

5.2.1. Captive bank vole colony used in study

All subjects included in the study were bank voles (*Myodes glareolus*). This research adhered to all guidelines for the use of animals in research outlined by the Study of Animal Behaviour/Animal Behaviour Society [421]. The work satisfied institutional guidelines and all legal requirements of the United Kingdom, where the work was completed. No specific licenses were required.

5.2.1.1. Management of captive colony

The bank voles used in the study were bred in the laboratory. They are first- and second-generation descendants of wild caught bank voles that were trapped in Cheshire (UK). I periodically trapped wild bank voles to maximise genetic diversity within the captive population.

Bank voles may develop diabetes [422]. Both wild-caught and lab-bred individuals have been found to develop diabetes in the captive population maintained in the laboratory. I tested all individuals in the captive population for diabetes to ensure that they were healthy. To test for diabetes, I took urine samples for each individual and used Diastix™ reagent strips for urinalysis (Bayer, Germany) to test for the presence of glucose. Any animals that tested positive for diabetes were culled. This significantly limited the number of individuals available

for tests. The numbers of males were particularly affected, as the prevalence of diabetes was greater in males than in females.

5.2.1.2. Indoor housing conditions

Indoor housing conditions were identical for all subjects throughout life. Cage floors were covered in substrate (Corn Cob Absorb 10/14 substrate). The substrate was sufficiently deep to enable digging, but not to allow complete submergence of individuals. Food and water were provided *ad libitum*. Animals were maintained on a reversed photoperiod (dark 8hr; light 16hr; white lights on 1700hr). Cardboard tubes and/or boxes were included in each cage as enrichment and shelter. All contact with animals was completed during the dark period. The temperature was maintained at 20±1^{O}C.

5.2.2. Preliminary experiments; the use of water to investigate traits related to dispersal behaviour

Experiments to examine dispersal behaviour were conducted either under controlled laboratory conditions or in a large outdoor enclosure (25m x 40m). Both male and female bank voles are capable of moving distances that greatly exceed the space available [316,460]. Dispersal is costly [24,142,192,319], and these costs are difficult to replicate in laboratory or semi-natural conditions. Previous studies have used passageways or barriers to examine the dispersal propensity of individuals [389,459,464–469]. Individuals that moved through such passageways or barriers were typically considered to be dispersers, particularly if the movement was completed repeatedly or was not reversed [389,459,464–469].

For terrestrial animals, movement through water is generally considered to be more costly than travelling over a solid surface. Water bodies are often considered barriers to emigration, a key part of dispersal movements, from a given area [319]. Several previous studies have used water filled chambers as barriers to study emigration in rodent species both in laboratory conditions [469] and in outdoor enclosures [467]. In order to use water to study dispersal in bank voles, it was critical to first establish that bank voles would cross water and that water represents a barrier to movement, as indicated in previous studies [319].

Two types of preliminary tests were completed to assess whether water represented a barrier to movement in bank voles. One of these tests included habituation to water, and the other did not. In each test, I compared the time taken to cross a chamber when left empty (i.e. a solid surface) and when filled with water. Water could be considered a suitable barrier

if bank voles were less willing to cross it compared to a solid surface. Here I outline the methods used in preliminary tests.

5.2.2.1. Subjects used in preliminary studies

Tests were completed using seven males and twelve females. All individuals were included in the first preliminary test (Section 5.2.2.3.), but only females were used in the later preliminary test (Section 5.2.2.4.). Individuals were ~6 months old at the time of the first set of preliminary tests and ~7 months old in the second test.

Each individual was singly housed in an M3 cage (48 x 15 x 13cm, North Kent Plastic cages Ltd., UK) before tests. No subjects had previously mated. All individuals were housed in the same room before and after the study. During the study period, animals were housed in the room in which tests were conducted.

5.2.2.2. Equipment used in preliminary tests

A piece of equipment referred to as a 'water unit' was used in tests. A schematic diagram of the water unit is shown in Figure 5.1. I placed the water unit in a larger enclosure (1.2m x 1.2m x 0.7m (l x w x h)) to ensure that any animals that escaped from the water unit remained contained. Perforated, transparent Perspex sheets were used to cover the water unit. These sheets prevented escape whilst enabling the entry of air and the observation of movements during tests.

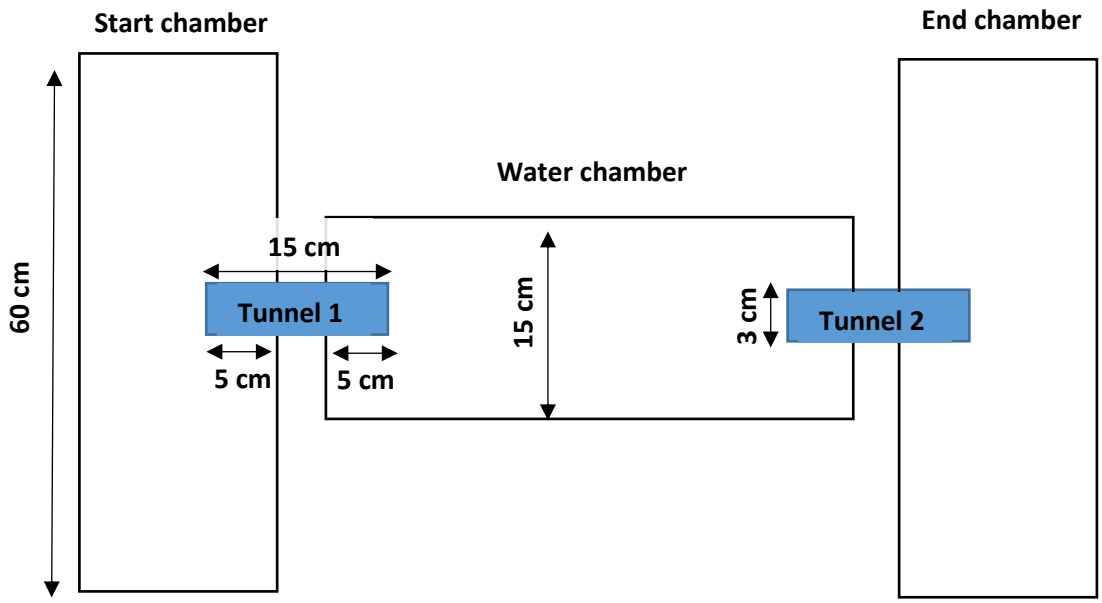

Figure 5.1. Schematic diagram of the water unit. The boxes with black outline represent chambers which the subjects can enter and explore freely. The blue boxes represent transparent circular tubes that allow individuals to move between chambers. The labels above each of these chambers and labels on tunnels indicate the respective names for reference. The water chamber can be filled with water or left empty; the start and end chambers are never filled with water. Measures on arrows represent dimensions of the equipment. The equipment is 12cm in height. The tubes are 6cm from the bottom floor of each chamber. The chambers comprise of terracotta coloured plastic planters and the tubes comprise of 3mm thick transparent Perspex cylinders.

5.2.2.3. Preliminary experiment including habituation periods

In previous studies individuals were not habituated to water before it was used as a barrier to movement [469]. Habituation was considered unnecessary as water naturally occurred in suitable habitats [469]. However, without habituation to water in this study, it would not necessarily be possible to determine whether hesitation to cross water relative to a solid surface occurs because it acts as a barrier to movement or because of neophobia. Here, I allowed habituation to exclude neophobia as a cause of increased hesitation to enter water.

In this experiment, individuals were placed in trials for 30 minutes on 4 consecutive days. An infrared camera was used so that trials could be viewed in real time in a separate observation room, and recorded for later analyses. The camera was suspended from above, and positioned centrally above the experimental equipment. Individuals were placed in the

centre of the start chamber at the beginning of each trial. The water chamber remained empty on days 1 and 2 of the experiment and filled 6cm deep (i.e. to the level of the bottom of connecting tubes) with room temperate water on days 3 and 4. The depth of water was sufficient to ensure that animals swam, rather than waded, across water. Days 1 and 3 were 'habituation days', subjects were given 30 minutes to habituate to the equipment and to the presence of water in the equipment, respectively. Days 2 and 4 were 'test days', and animals were removed from the equipment either after 30 minutes or when animals reached the end chamber. See Figure 5.1 for a diagrammatic representation of the relative locations of chambers and connecting tubes.

I did not test all 7 males and 12 females in the same trial period. Instead, I tested animals in sets of 4 or 5 individuals. I balanced the sex, age and relatedness of animals between trial periods. The individuals included in a trial period were moved to the room in which tests were conducted 24 hours before the testing period began, and were maintained in this room throughout the trial period.

Individuals were placed in trials in the same order on each day of the trail period that they were included in. The first test on each day commenced 30 minutes after the beginning of the dark period. The water unit, Perspex sheets used to cover the equipment and handling tubes used to transfer animals between the home cage and water unit were cleaned with 70% ethanol both before trials commenced and between individuals.

5.2.2.4. Preliminary experiment without habituation

The earlier preliminary test with habituation was conducted to minimise the likelihood that any apparent increase in the time taken to cross water, rather than a solid surface, was due to neophobia. However, in that earlier study, individuals experienced the water chamber for short periods on four consecutive days without reward. This could mean that individuals were less likely to enter the water, which was only present later in the trial period, due to decreased motivation to explore the equipment. I therefore completed a shorter test to eliminate this possibility and confirm that water is a suitable barrier for this study.

In this test, the procedure used was similar to that used in tests with habituation (see Section 5.2.2.4). However, the two habituation days were eliminated, such that the individuals were placed in tests only on two consecutive days. On the first day of a trial period, the water chamber was left empty, but it was filled with water on the second day. Individuals were removed from the water unit (Figure 5.1) after 30 minutes or after reaching the end chamber on both days.

5.2.2.5. Can water be used as a barrier to study dispersal in bank voles?

I completed video analyses to assess the relative hesitance of individuals to enter the water chamber when water was present and absent. Hesitation to enter the water chamber was measured in two ways; number of attempts at entry made before entry and length of time before entry to the water chamber. An attempt at entry included any protrusion of at least the nose of an individual out of Tunnel 1 (see Figure 5.1) that was not followed by entry into the water chamber. An entry included any purposeful movement into the water chamber. Purposeful movements included any movement that included an attempt to traverse the water chamber. When the water chamber was filled, individuals often attempted to get on top of the tunnel or to reach the lid rather than entering the water. As a result, individuals sometimes fell backwards into the water. Backwards entries into the water were not counted as entries. I used one-way paired Wilcoxon tests to determine whether bank voles exhibited greater hesitance to enter the water chamber when water was present compared to when the chamber was empty. Non-parametric tests were used because the data did not satisfy the requirements of parametric equivalents. I used one-way tests as I expected subjects to exhibit greater hesitance to enter the chamber when water was present. If a subject failed to cross, then the time to cross was recorded to be 30 minutes (the maximum length of tests).

In the preliminary tests using habituation, I compared data from test days when water was absent and present (i.e. days 2 and 4 of trials, respectively). I found that bank voles made significantly more attempts to enter the water chamber before trying to cross the chamber when water was present (average number of attempts when water was absent (±se) = 0.05(±0.05); average number of attempts when water was present (±se) = 7.05(±1.06)) (one-way paired Wilcoxon test; n=19, V=190, P<0.001). Subjects also took significantly longer to enter the water chamber when water was present (average time until entry when water was absent (±se) = 179.21(±48.23) seconds; average time until entry when water was present (±se) = 1037.00(±153.88) seconds)) (one-way paired Wilcoxon test; n=19, V=190, P<0.001).

In preliminary tests without habituation, the number of attempts to enter the water chamber was significantly greater when the chamber was filled with water (average number of attempts to enter the chamber when water was absent (±se) = 0.33(±0.19); average number of attempts to enter the chamber when water was present (±se) = 7.08(±1.33)) (one-way paired Wilcoxon test; n=12, V=75.5, P=0.002). The length of time to enter the water chamber was significantly greater when water was present in the chamber (average time until entry when water was absent (±se) = 367.50(±115.02) seconds; average time until entry when

water was present (±se) = 767.92(±152.83) seconds)) (one-way paired Wilcoxon test; n= 12, V=69, P=0.008).

Taken together, these results suggest that crossing water is relatively costly for bank voles compared to travelling on a solid surface. This indicates that water can be used as a barrier to enable studies on dispersal behaviour in bank voles in a similar manner to previous studies on rodent species (e.g. [467,469]).

5.2.3. Investigating maternal effects on dispersal behaviour

5.2.3.1. Subjects used in maternal effects study

Subjects were born under simulated conditions of either 'high' or 'low' levels of social competition. The procedure used is outlined in Chapter 4 (Section 4.2.2.) and is similar to that used previously in male bank voles (*M. glareolus*) [102] and house mice (*M. musculus*) [424]. Briefly, adult females were presented with odours from either one or three conspecific females. The odour cues were supplemented with exposures to the females from which scents were taken. The odour treatment was applied from before conception of the young until all offspring were weaned, whereas direct exposure to conspecific females only occurred prior to pregnancy. The juveniles subsequently produced by these females were used to assess maternal effects on dispersal behaviour.

The study occurred in two blocks (Section 4.2.2.), so pups were produced in two distinct sets. In total, 16 litters were produced during the study, and 15 survived until weaning (6 litters from the first block, 9 litters from the second study block). Thirteen male bank voles were weaned from the first study block (2 high competition, 11 low competition) and sixteen males were weaned from the second (11 high competition, 5 low competition). Ten female bank voles were weaned from the first block (5 high competition, 5 low competition) and nineteen were weaned from the second (17 high competition, 2 low competition).

Pups were housed with mothers in MB1 cages (45 x 28 x 13cm, North Kent Plastic cages Ltd., UK) until they were weaned at 28 days of age. All offspring were pit-tagged (RFID tags, Francis Scientific Instruments, UK) before weaning. Male offspring were weaned individually into M3 cages and maintained in a separate animal room. Female offspring were released in semi-natural enclosures alongside their mothers (see Section 5.2.4.1. for details on semi-natural enclosures).

The offspring in different blocks were produced approximately 3 months apart. Investigations of dispersal behaviour were thus completed in two sets 3 months apart.

5.2.3.2. Maternal effects on dispersal behaviour in males

I assessed the dispersal propensity of males indirectly by investigating boldness. Dispersal propensity is associated with boldness (see e.g. [231]), with bolder individuals generally expected to be more likely to disperse. I expected that the offspring of females subject to cues indicating high competition would be more likely to disperse, and thus bolder, than males born in the low competition treatment group.

I used the water unit (Figure 5.1) to assess the relative boldness of individual males born in each treatment group under laboratory conditions. The water chamber was filled 6cm deep with room temperature water during each trial. I considered entry into water to be a risk to males, particularly because males had no prior experience with water or the equipment. Bolder males were expected to be more willing to enter the water.

The boldness, and thus likely dispersal propensity, of males was assessed during a single trial that was completed during the dark period (see Section 5.2.1.2. for details on the lighting schedule used during the study). Tests were conducted when males reached 56 days (± 1 day) of age. Male bank voles are expected to reach sexual maturity at approximately 42 days of age [429], thus males should have been sexually mature at the time of the study. The slight deviation in age at the time of testing occurred because it was not possible to test all males on the same day. The relatedness, age and treatment group of origin were balanced across days.

At the beginning of each test, I placed males individually in the centre of the start chamber of the water unit. Males were left in the unit for 30 minutes or until they entered the end chamber. Males were returned directly to their home cages after trials. I used an infrared camera suspended above the water unit so that I could monitor trials in real time in a separate observation room and to record trials. Males were moved between their home cage and the water unit using a Perspex handling tube. All equipment was cleaned with 70% ethanol between individuals.

5.2.3.3. Maternal effects on dispersal behaviour in females

5.2.3.3.1. Outdoor semi-natural enclosures

The dispersal propensity of females was assessed in a large outdoor semi-natural enclosure (40m x 25m) within the Leahurst Campus at The University of Liverpool. The lower 1.2m of the enclosure walls comprised of solid Aluzinc, and the upper 0.8m consisted of wire mesh which extended across the roof of the enclosure to prevent predation and escape. There was

a small foyer area (2m x 2m) at the entry to the enclosure. There was a second door leading to the enclosure from the foyer. The double door entry system was intended to minimise the risk of escape. There was a paving stone path (approx. 70cm wide) around the outer edge of the enclosure. The remainder of the area comprised of vegetation that occurred naturally in the area. A schematic layout of the enclosure is provided in Figure 5.2.

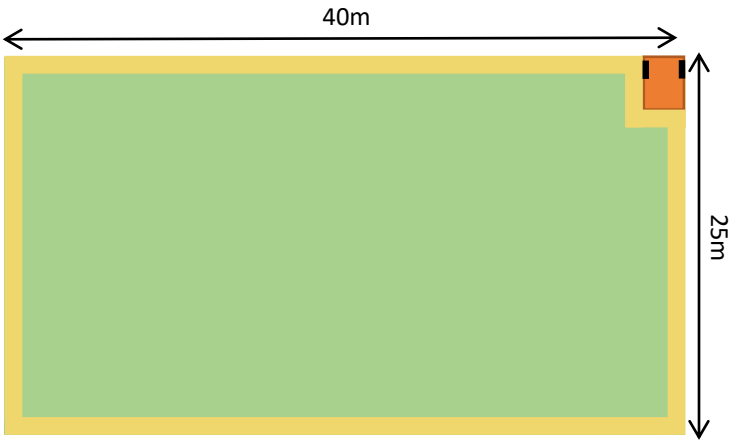

Figure 5.2. Schematic layout of the outdoor semi-natural enclosure. The area containing vegetation is represented in green. The paving stone pathway (0.7m wide) is depicted in gold. The foyer is shown in orange. Positions of doors are indicated using black boxes. The size of the sides of the enclosure are shown on black arrows.

Before the study, the enclosure housed 30 lab-born house mice (*Mus musculus*) which had been released into the enclosure, and an unknown number of wild field voles (*Microtus agrestis*). I conducted trapping daily for 2 months prior to the start of the study to clear the enclosure. Trapping was conducted using 6 handmade one-way door traps (built by Liane Hobson), 40 Longworth traps (nhbs, UK) and up to 100 tube traps (TubeTrap, BioEcoSS Ltd., UK). When there was no evidence of animals in the enclosure for a week, I added feeders containing small amounts of food. Food was not taken for 2 weeks prior to the start of the study. There was no evidence that any house mice or field voles remained in the enclosure during the experiment.

I did not manage the vegetation in the enclosure whilst subjects were present because this could have influenced results. The vegetation became extremely overgrown in the first study block, and became dominated by stinging nettles (*Urtica dioica*) and thistles (a species within the tribe Cynareae, likely the milk thistle, *Silybum marianum*). At the end of the first study period, the height of vegetation in the majority of the enclosure exceeded 1.6m, making

working conditions difficult. The vegetation was managed by selectively removing stinging nettles and thistles to return the composition of vegetation to a similar condition to that at the beginning of the first study period. Habitat management occurred over a five-week period between study blocks.

5.2.3.3.2. Release of female family groups to the enclosure

To examine potential maternal effects on dispersal in female bank voles, I released adult females in the outdoor enclosure alongside their female offspring (hereafter referred to as 'female family groups'). The study was completed in two seperate blocks (Section 4.2.2.), with subjects from each block present in the enclosure for a seven-week study period. Subjects from the first study block were present in the enclosure from 2nd May – 13th June 2015 and those from the second block were present from 15th August – 3rd October 2015. Subjects were recaptured at the end of each period and returned to indoor housing.

Female family groups were moved to the outdoor enclosure from indoor housing in their home cage. A nest box (hamster igloo (approximately semi-circular, maximum measurements 14 x 11 x 13cm), Savic, Belgium) had been provided for each litter when offspring reached one week of age (Figure 4.2.4.1.). I released subjects by guiding them into this nest box within their cage, before moving this nest box under upturned RM2 cages at release sites. This method was used in an attempt to ensure that family groups remained together immediately after release. Photographs of the release procedure are shown in Figure 5.3.

An upturned RM2 cage was positioned at each selected release site for shelter. Hay was placed under the cage along with some food (LabDiet 5002 Certified Rodent Diet, Purina Mills, USA). The shelter was held down using a house brick. A food box was provided under a shelter beside the cage which comprised of three breeze blocks. Each food box consisted of a water tight food container and could be entered through a circular transparent Perspex tube which was 15cm in length and 3cm in diameter. This equipment is depicted in Figures 5.3. and 5.4. The food box is visible in Figure 5.3, but is shown more clearly in Figure 5.4.

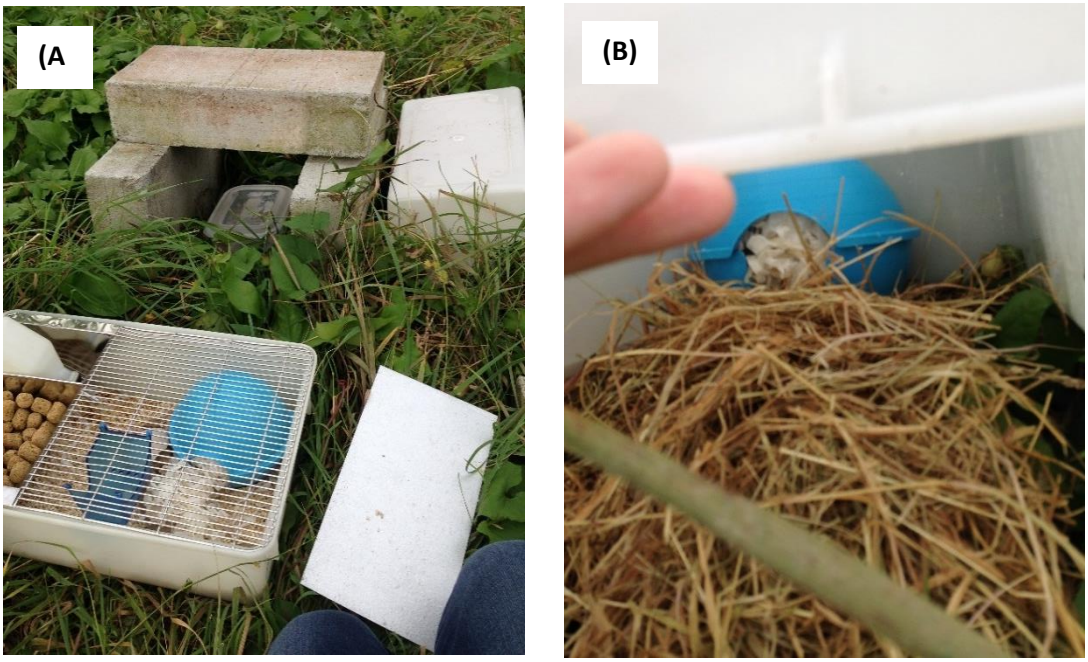

Figure 5.3. Photographs depicting the release procedure. (A) MB1 cage containing a female family group beside randomly selected release location with all necessary equipment in place. (B) Nest box containing a female family group underneath upturned cage. The blue semi-circular box in each picture is the next box.

Figure 5.4. Food box filled with LabDiet pellets and with Perspex tunnels to allow entry/exit of subjects.

Three water dispensers were equally spaced down the centre of the enclosure. Water dispensers were checked daily, refilled as necessary and cleaned every two weeks. An excess of food (LabDiet 5002 Certified Rodent Diet, Purina Mills, USA) was provided in food boxes. Food boxes were checked once daily. Pellets were removed if they become wet. Excess moisture in food boxes was primarily caused by the presence of gastropod species. The enclosure contained high densities of gastropod species, which often entered food boxes. All gastropods were removed daily from food boxes, which were cleaned weekly. Additional food, including invertebrates and vegetation, occurred naturally in the enclosure.

A maximum of 16 litters could have been produced in each study block (see Section 4.2.2.). As such, sixteen potential release locations were planned in the enclosure. The positions of potential release locations were planned to maximise the distance between them and the edges of the enclosures. Release locations were ~4.4m apart on the longer (40m) side of the enclosure and ~8.3m on the shorter (25m) side. The relative positions of potential release locations are shown in Figure 5.5. A release location was selected at random for each female family group. Equipment was only placed at release locations site if it was selected for a female family group.

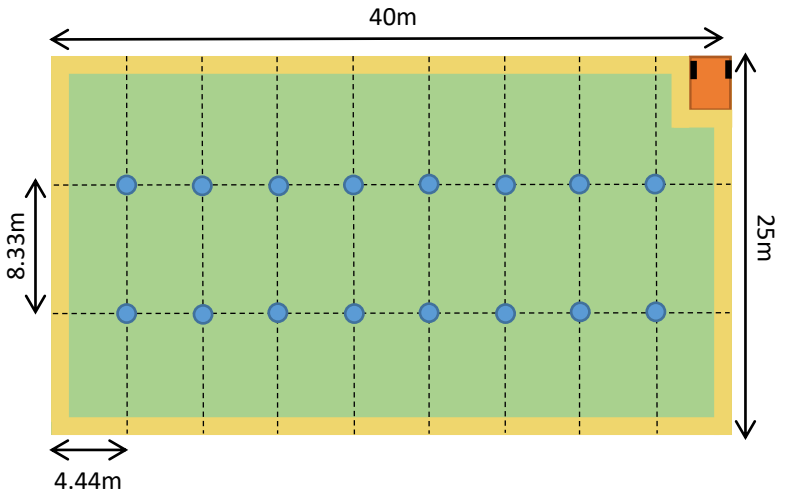

Figure 5.5. Layout of semi-natural enclosure including potential release locations. The area containing vegetation is represented in green. The paving stone pathway (0.7m wide) is depicted in gold. The foyer is shown in orange. Positions of doors are indicated using black boxes. Black dashed lines represent equal measures of distance between potential release sites, not features of the enclosure. The size of the sides of the enclosure and the distances between release locations are shown on black arrows. Blue circles indicate the positions of potential release locations.

5.2.3.3.3. Male scents

Males were not released in the enclosure to prevent breeding. However, the absence of males in a habitat is atypical. To prevent abnormal behaviour in females due to the absence of males, I used scent samples to simulate the presence of males in the enclosure. Scent samples comprised of approximately 12.5g soiled sediment taken from the cages of individually housed male bank voles that were unrelated to female subjects in the study. Samples were only acquired from non-diabetic males. Scent samples were taken from four sexually mature males in each study block. The four males used differed between study block.

The enclosure was divided into four equal 'male territories'. Four odour samples were taken from each male every day of the study. The four odour samples from a male were randomly distributed across the 'territory' of that male. I took measures to ensure that I did not introduce bias in the distribution of male odours. I divided each territory into 10 equal sections. Each section was assigned a number and four numbers were selected at random each day. I placed the odour sample at random within the four selected sections. A diagram of how the enclosure was subdivided is shown in Figure 5.6. The odour samples were placed in a glass tea light holder. The glass containers were placed inside a square plastic plant pot (10cm x 10cm x 15cm) to protect the scent sample from wind and rain. The plant pots were turned on their side, with one side open to allow females to gain access to the scent sample. Odour samples were refreshed daily.

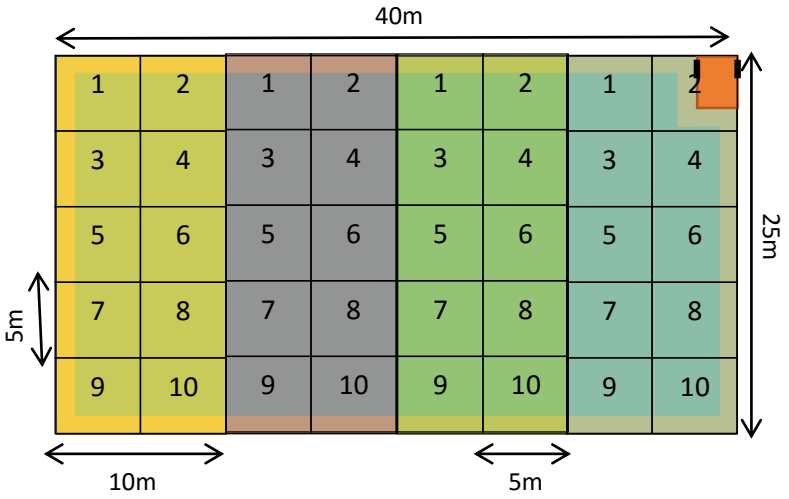

Figure 5.6. Subdivision of the semi-natural enclosure into male territories. The blue, green, purple and yellow translucent rectangles represent the 'territories' of four distinct males. A 'territory' is an area in which the scent of a particular male can be placed. The layout of the enclosure is visible through these rectangles, lighter areas indicate a pathway (0.7m wide) and darker areas represent vegetation. The numbers indicate the number assigned to each of the 10 sections within each territory. Scents were not placed in the foyer area, represented by an orange square.

5.2.3.3.4. Monitoring dispersal behaviour in the enclosure

The dispersal propensity of female offspring was assessed using 'dispersal units'. Dispersal units consisted of two chambers which were identical to those used in the preliminary experiments (Terracotta plastic planters (12cm x 60cm x 15cm (h x l x w))). The first chamber was filled 6cm deep with water, the second was not. Individuals were able to enter the first chamber and move between chambers using circular Perspex tunnels (10cm in length, 3cm in diameter) identical to those allowing entry to food boxes. Tunnels were positioned between 6cm from the floor of the equipment. A wooden ramp was provided to facilitate entry to a dispersal unit. The chambers and ramps were stabilised using compost. A partly submerged platform was provided beside the tunnel at the end of the water chamber. Platforms comprised of a sponge with a scouring pad on top tied to a small, square plastic plant pot (10cm in length and width and cut to be 4cm in height). The scouring pad formed the top of the platform to maximise the potential for animals to grip the surface. The platform was secured to the bottom of the water chamber using electrical tape.

A closed-faced Ugglan trap (Ugglan special number 1, Grahnab, Sweden) was placed around the tunnel leading to the end chamber to act as a one-way door. The trap allowed animals to

enter the end chamber, but the trap mechanism prevented re-entry to the water chamber. Each trap was placed on top of trap covers. Trap covers were designed to fit over the top of Ugglan traps and cover the top of the trap and the top ~3cm of the sides of the trap. The resultant space beneath traps provided additional shelter for voles.

The end chamber was provisioned with food pellets (LabDiet 5002 Certified Rodent Diet, Purina Mills, USA), hamster food (Harry hamster muesli, Supreme, UK), apple chunks (to prevent dehydration) and hay (nesting material). Both the water and end chambers had 10 holes (~1cm in diameter) drilled 2cm from the top of the chamber to allow the circulation of air. One animal in the first set was able to escape after chewing the plastic to expand these holes. All holes in the end chamber were subsequently covered with wire mesh to prevent any further escapes. The dispersal units were covered with transparent Perspex sheets. The top of each Perspex sheet was covered with black electrical tape to provide cover. A schematic diagram of the equipment is shown in Figure 5.7, and a photograph of the equipment *in situ* is shown in Figure 5.8.

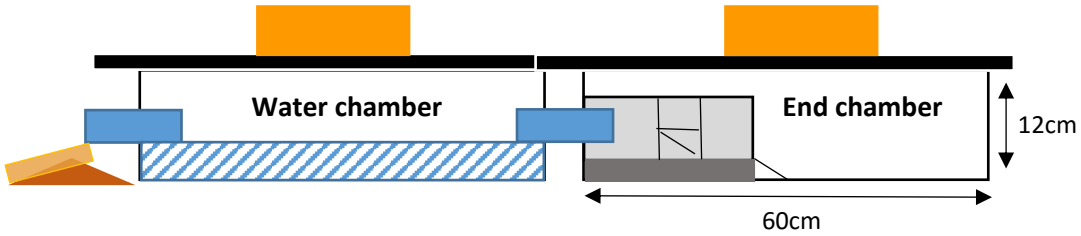

Figure 5.7. Schematic diagram of a dispersal unit. The water and end chambers consist of terracotta coloured planters. Blue boxes represent transparent plastic tunnels that allow movement between chambers. The bottom of each tunnel is 6cm from the base of the unit. The water chamber is filled 6cm deep with water (represented by blue striped area). The light grey box represents a closed-face Ugglan trap, lines within the box represent the trap mechanism and the line out of the box represents the end door which acted as a ramp. The dark grey box represents the cover for the Ugglan trap. The wooden ramp (light brown rectangle) to enable entry to the unit was supported by compost (dark brown triangle). The equipment was covered with 5mm thick Perspex sheets (20cm x 90cm) (black rectangles) covered in black electric tape. The sheets overlap over the unit. The Perspex sheets were held in position with house bricks/breeze blocks (orange rectangles).

Figure 5.8. Photograph of a dispersal unit *in situ* in the semi natural enclosure.

Nine dispersal units were used. All dispersal units were positioned around the edges of the enclosure. The positions of dispersal units in the enclosure are shown in Figure 5.9. I checked dispersal units twice daily; at the beginning and end of each day. Any individuals found in dispersal units were removed using a plastic handling tube. The location, weight and identity of any captured animals were recorded before release beside the dispersal unit. I was blind to the treatment group of animals when identified using pit-tag numbers. As well as checking for the presence of animals, I also monitored the water levels in dispersal units to ensure that they remained unchanged. Water was refreshed weekly when dispersal units were cleaned.

5.2.3.3.5. Monitoring movements of females in the enclosure

The movements of animals in the enclosure can be associated with dispersal propensity. Exploratory behaviour is positively linked with dispersal [470,471]. Individuals with a higher level of motivation to attempt to disperse may be more likely to move greater distances in the enclosure. The position of an animal in the enclosure may also be linked to dispersal behaviour. For example, animals attempting to move out of an enclosure are more likely to spend time near the outer edge of the area. Indeed, several studies, including the present

one, positioned equipment to monitor movement out of an area on the outer edges of the site/enclosure (e.g. [389,472]).

I monitored the movements of individuals throughout the seven-week long study period using Longworth traps (nhbs, UK). I used a total of 36 Longworth traps in transects across the enclosure to measure home range, movement distances and location in the enclosure. The six transects were placed 8m apart along the long side (40m) of the enclosure. Six Longworth traps were placed 5m apart on each transect. The foyer area prevented one trap from being positioned on a transect, this trap was placed as close as possible to the intended position. The layout of Longworth traps is shown on Figure 5.9. Longworth traps were deployed continuously for two days for each week of the study. All traps were checked before each use to ensure functionality. Each Longworth trap was provisioned with hay for nesting material, apple chunks to prevent dehydration, and food (LabDiet 5002 Certified Rodent Diet, Purina Mills, USA and Harry hamster muesli, Supreme, UK). Traps were checked every 3 hours during 12 hours of daylight, and were left unchecked for 12 hours overnight. If the trap door was closed, this indicated that the trap was triggered. Triggered traps were emptied into a transparent plastic bag. If an animal was present in the trap, the identity, weight and location of the individual was recorded before the vole was released in the same location. The provisions in a trap were refreshed after the release of an animal. Traps were cleaned at the end of each trapping period.

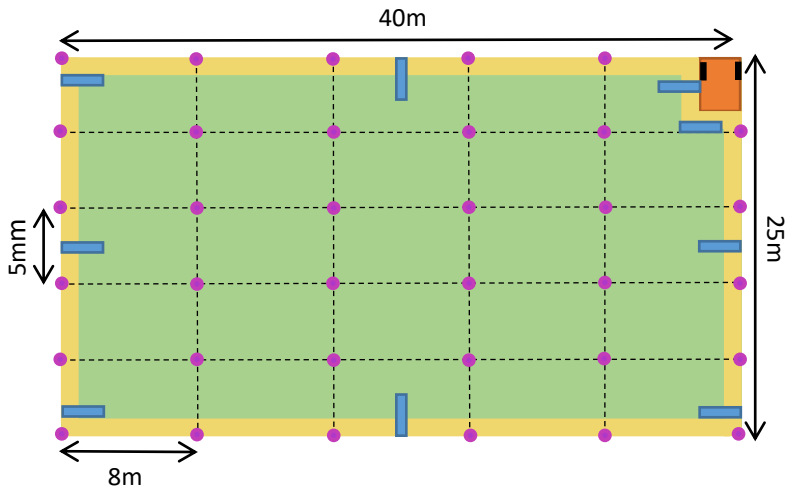

Figure 5.9. Schematic diagram indicating the positions of dispersal units and Longworth traps in the enclosure. The vegetation (green area) in the enclosure is surrounded by 0.7m wide path (gold lines). A foyer (orange rectangle) is present at the entrance of the enclosure. Doors are represented by black rectangles. The positions of Longworth traps (when deployed in the enclosure) are indicated by purple circles. Dashed black lines are used to indicate the spacing of traps. Dispersal units (blue rectangles) are continually present in the enclosure throughout each study period. Measurements are indicated on black arrows.

5.2.4. Data processing

5.2.4.1. Investigating potential maternal effects on male dispersal behaviour

5.2.4.1.1. Video analyses

I used video recordings to quantify the length of time before male bank voles entered the water chamber and end chamber (see Figure 5.1 for details on the water unit used for tests). If males did not enter these chambers during trials, I recorded the time until entry as 30 minutes (i.e. the maximum length of trials). Behaviour was recorded blind to the treatment group into which each subject was born.

5.2.4.1.2. Statistical analyses

I used linear mixed effects models to assess the effect of treatment on the time to enter the water and end chambers of the water unit. I included the body weight of males and the treatment group of origin as fixed effects. Body weight was included as previous studies have shown that heavier animals may be more able to cope with the costs of dispersal [457,470], so they may enter the water and end chambers more quickly. I included the study block and litter in which individuals were born as random effects. I termed models that included all

factors as 'full models'. To identify whether there is a maternal effect due to treatment group, I compared a full model to a model that did not include treatment group as a factor (hereafter referred to as the 'reduced model'). The models were compared using a likelihood ratio test. If there was a significant difference and the log-likelihood values indicated that the full model was more likely, then it suggests that there was a significant effect of treatment group on the behaviour of male bank voles.

5.2.4.2. Statistical analyses to assess maternal effects on dispersal behaviour in females

5.2.4.2.1. Data from dispersal units

In total, only three voles (1 female from each treatment group in the first block, and 1 low competition female from the second block) entered dispersal units during the study. All females that entered dispersal units did so for the first time seven days after release. Both low competition treatment group females that entered dispersal units were only trapped in dispersal units once. By contrast, the high competition treatment group female was found in the dispersal unit at least once per day for the last 6 weeks of the study period it was in the enclosure (i.e. the first study block). Overall, there were insufficient data on behaviour in dispersal units to conduct statistical analyses.

5.2.4.2.2. Data from trapping females

I used several measures in order to compare the movements of females in the enclosure, as a means of making inferences about their likely dispersal behaviour. Specifically, I considered the home range size of females, the maximum distance between consecutive traps, the level of overlap with maternal home range and the relative number of captures near the outside of the enclosure (see Section 5.2.3.3.5 for details).

High levels of social competition should drive more dispersive behaviour (i.e. greater dispersal propensity and/or distance) [24,141,142]. Thus, I expected, if maternal effects acted adaptively on dispersal behaviour, females born in the high competition treatment group would exhibit traits related with greater dispersal propensity and/or distance.

Home range size has previously been associated with dispersal behaviour in mammalian species, with animals with larger home ranges typically dispersing further [473–475]. Indeed, home range size is typically a better predictor of dispersal distance than body size [473]. I also expected that individuals that are more likely to disperse would move away from their natal area, so their home ranges would overlap less with those of their mothers. More dispersive individuals may also be more mobile, I therefore anticipated that such individuals

would move longer distances between consecutive captures. Lastly, I expected individuals that had a higher dispersal propensity would spend relatively more time near the outer edge of the enclosure, as they would be more likely to attempt to move out of the area. Thus, such animals should be trapped more frequently on the outer edge rather than in other areas of the enclosure.

I calculated the home range size of females, the maximum distance between consecutive traps and the level of overlap with maternal home range by mapping trapping data on to a scale computer model of the enclosure in QGIS v 2.8.1. [476]. Home range size was defined by the locations of the outer-most points at which the subject was captured. Individuals may have been caught in other traps within that area, but these did not define the maximum range of the bank vole. The degree of overlap with the maternal home range was approximated by first calculating the home ranges of adult females and their offspring and measuring the area of the overlap between the two. I measured the distance moved between the sites of each consecutive capture, I took the longest of these distances to be the maximum distance between consecutive captures. All calculations of area and distance were completed using the measure tool in QGIS v 2.8.1. [476].

Home range could only be calculated if individuals were captured in at least 3 different traps; this was the minimum adequate to encompass an area. One mother from the low competition treatment group in the first study block was never captured after release and was presumed dead. It was thus not possible to determine the level of overlap with maternal range for the two female offspring of that subject. A high competition mother was found dead 9 days before the end of the first study block. There were sufficient data to calculate the home range of this female, and no data were excluded as a result. In the second block, one high competition offspring was not captured until after the study period had finished. A second, unrelated offspring from the high competition group was only captured in two traps. Whilst there were sufficient data for this female to calculate the maximum distance moved, home range could not be calculated.

5.2.4.2.3. Statistical analyses of trapping data

I used linear mixed effects models to determine whether there was evidence of an effect of social competition in the maternal environment on offspring dispersal behaviour. In all full models I included study block and the litter in which subjects were born as random effects.

In models of home range size, overlap with maternal home range and maximum distance moved, I included treatment group, total number of captures and average body mass as fixed

effects. The total number of captures was included to account for any potential effect of trappability of subjects on these measures. I recorded the body masses of females at every capture (see section 5.2.3.3.4), and these generally increased over the study period. I therefore used the average mass of females recorded throughout the study in models. In tests considering the overlap with maternal home range, I additionally included the maternal home range size as a fixed effect. This was included as larger overlaps are more likely when mothers have greater ranges. I included the number of females in each family as a fixed effect in all models. Females in smaller family groups may be less likely to disperse as they may experience relatively low levels of local resource competition.

Models investigating the relative number of captures on the outer edge of the enclosure were structured slightly differently. I used the number of captures in traps/dispersal units on the outer edge of the enclosure as the dependent variable, and included the total number of captures and treatment group as fixed effects. I included the litter and block of origin of each animal as random effects.

In each analysis, I used likelihood ratio tests to compare models including treatment as a factor (i.e. a full model) to models excluding treatment group (i.e. a reduced model) to assess the impact of treatment group of origin on female movements within the enclosure.

5.3. Results

5.3.1. Do maternal effects influence behaviours related to the dispersal of males?

I measured the time taken for males to enter both the water and end chambers of the dispersal unit. I expected that males born in the high competition group would enter each chamber more quickly. However, there was no significant difference in the time taken to enter water between treatment groups (high competition males: average(±se) = 1074(±151) seconds, low competition males: average (±se) = 1140(±109) seconds, n= 29, log likelihood full model = -218.57, log likelihood reduced model = -218.58, χ^2 = 0.010, Df = 1, P = 0.9193). Similarly, there was no significant difference in the time taken to enter the end chamber between treatment groups (high competition males: average(±se) = 1463(±128) seconds, low competition males: average (±se) = 1306(±101) seconds, n= 29, log likelihood full model = -215.28, log likelihood reduced model = -215.63, χ^2 = 0.703, Df = 1, P = 0.402).

Taken together, these results indicate that the social competition experienced by female bank voles does not influence the behaviour of their male offspring.

5.3.2. Do maternal effects influence behaviours related to the dispersal of females?

I used several measures to indirectly assess the relative natal dispersal behaviour in female bank voles born in different treatment groups. Specifically, I considered the relative number of captures on the outer edge of the enclosure, home range size, the maximum distance between consecutive captures and the level of overlap with maternal home ranges.

Females born in the high competition treatment group were expected to be more likely to disperse. Thus, I expected to capture such individuals more frequently on the outer edge of the enclosure. Against this hypothesis, I found no difference in the number of captures on the outer edge of the enclosure between groups (average proportion of captures on the outer edge, average (±se): high competition = 0.728(±0.254), low competition = 0.608(±0.328); n= 28, log likelihood full model = -13.85, log likelihood reduced model = -13.91, χ^2 = 0.118, Df= 1, P = 0.732).

Individuals with larger home ranges were considered more likely to disperse longer distances, so I expected that females born in the high competition treatment group to have larger home ranges. However, I found no evidence of a difference in home range size between treatment groups (home range size, average (±se): high competition = 458.09(±53.92)m^2, low competition = 507.80(±80.54)m^2; n = 27, log likelihood full model = -177.41, log likelihood reduced model = -177.77, χ^2 = 0.703, Df = 1, P = 0.402).

Females that travelled a longer distance in a single movement were thought to be more likely to disperse, and high competition females were expected to be more motivated to leave the enclosure. Contrary to this prediction, there was no difference in the maximum distance moved between consecutive captures between treatment groups (distance moved between consecutive captures, average (±se): high competition group = 32.50(±1.56)m, low competition group = 31.82(±3.67)m; n = 28, log likelihood full model = -2,34, log likelihood reduced model = -1.22, χ^2 = 1.122, Df = 1, P = 0.289).

The level of overlap between the home range of an individual and that of their mother was expected to be lower in females with greater dispersal propensity. Thus, I expected the level of overlap with maternal home range to be lower in females born in the high competition treatment group. However, I found no significant difference between treatment groups (overlap with maternal home range, average (±se): high competition = 321.16(±57.04)m^2, low competition = 281.73(±135.07)m^2; n = 25, log likelihood full model = -154.10, log likelihood reduced model = -155.08, χ^2 = 1.944, P = 0.163).

5.4. Discussion

5.4.1. Maternal effects on dispersal behaviour in male bank voles

When there are more conspecifics in an area (i.e. when population density is relatively high), individuals are more likely to experience high social competition. Individuals can avoid the high levels of competition associated with high density environments by dispersing. I therefore expected male bank voles born in the high competition treatment group to be more likely to disperse and/or to disperse further than those born in the low competition treatment group. Contrary to initial expectation, I found no evidence of a difference in dispersal behaviour between treatment groups. This indicates that the social competition experienced by females does not cause a maternal effect on the dispersal behaviour in male offspring.

I considered boldness to be an appropriate measure of dispersal behaviour in male offspring. Several studies have found associations between behavioural traits, including boldness, and dispersal behaviour (reviewed in [231]). For example, the level of boldness is closely associated with dispersal distance in the Trinidad killifish (*Rivulus hartii*) [477]. Similarly, in a more extensive study, natal dispersal behaviour was closely associated with boldness in great tits (*Parus major*) [470]. Taken together, the results of such studies suggest that boldness typically promotes greater dispersal propensity and distance [231]. However, in some cases, bolder individuals may be less likely to disperse [230].

I assumed that measures of the time taken to enter the water and to travel across water (i.e. to enter the end chamber) were appropriate representations of boldness, and thus of dispersal propensity and distance. There is no reason to expect that these measures were inappropriate. Males had never experienced water or the equipment, and bolder individuals typically exhibit more exploratory behaviour in novel environments [231]. As such, bolder males are likely to enter different chambers more quickly. If the treatment instead caused the opposite effect, such that dispersal propensity was lower in bolder individuals, a difference in behaviour would still have been evident.

Any difference in the behaviour of males between treatment groups should have been evident in trials. Indeed, previous studies have used equipment similar to the water unit in this study to detect differences in behavioural traits [478,479] and in dispersal propensity [469] between individuals in rodent species. In each of those studies, as in this study (Section 5.2.2), individuals were found to take longer to cross water relative to a solid surface. This was likely to be because the costs of crossing water, both energetically and in terms of risk,

are relatively high. Swimming is energetically costly compared to running and walking [426,480]. Moreover, getting wet may influence the ability to remain warm, increase metabolism and can thus reduce the probability of survival. Swimming is also relatively slow and there is generally less shelter in water, potentially causing relatively high predation risk. The fact that crossing water may be relatively costly for bank voles is consistent with findings suggesting that it is a barrier to their dispersal [319].

Although the methodology in the present study is similar to that in previous studies, it is not identical. For example, the depth of water used here was shallower than in tests on *Mus spicilegus* and *Mus musculus* (6cm instead of 8cm) [469]. *M. spicilegus* and *M. musculus* were able to wade, rather than swim, in 6cm of water [478,479], but 8cm was sufficient to ensure swimming [469]. By contrast, in all observations in this study (n>50) bank voles consistently swam when water was 6cm deep. Thus, although the water depth here differs from that in other studies, it can be considered equivalent, and should not have impacted results.

Another difference compared to other studies is the length of tests. In this study, male behaviour was assessed in a single trial, but other studies have used longer observational periods. Groó *et al*. [469] tested natal dispersal behaviour in newly weaned *M. musculus* and *M. spicilegus* over 100 days. In that study, whole litters were placed into chambers without their parents. Animals that moved over a water basin on two consecutive days were considered to be dispersers, and others were 'residents'. I did not adopt this methodology due to space limitations and the risks of breeding and severe aggression between kin. The shorter time period employed in the present study could have reduced the likelihood of detecting a difference in likely dispersal propensity. However, any differences in boldness should have been evident in the present study. Indeed, differences in behaviour have been detected in relatively short-term tests using water as a barrier to measure motivation [478,479]. Male bank voles should have achieved sexually maturity before the tests commenced [429]. Previous studies have shown that male bank voles are sexually mature at emigration [316], so any differences in behaviours associated with dispersal should have been evident in the study. The method employed here does have an advantage over that used in Groó *et al*. [469], as it ensures that male dispersal behaviour is not affected by interactions between siblings.

Taken together, the evidence provided above suggests that the methodology used in tests did not preclude the detection of a difference in male bank vole behaviour. Instead, the absence of a difference likely reflects the lack of any maternal effect on dispersal behaviour

in male bank voles. There are several possible reasons for this, which are not necessarily mutually exclusive.

There is, to my knowledge, little direct evidence that maternal effects can influence boldness or dispersal behaviour in mammalian species. Indeed, several quantitative genetic studies have found that maternal effects on personality traits, including boldness, are negligible [481]. However, experimental studies have found more evidence that maternal effects may influence offspring personality traits [481]. For example, the components of the milk provided to young during lactation influences offspring behaviour in rhesus macaques (*Macaca mulatta*) [435]. There is also evidence from studies on mammalian species that behavioural phenotypes correlate with factors such as body size [482] which are related to dispersal behaviour [473] and can be influenced by maternal effects [263,269,411]. Although evidence from mammalian species is relatively scarce, there is considerable evidence for maternal effects on personality traits and dispersal behaviour in reptiles [250,389,399,446] and birds [395,396,452,453]. Thus, there is no reason to suspect that maternal effects could not influence boldness or dispersal in the bank vole, or in other mammalian species.

It is possible that, although social competition may cause maternal effects on dispersal, the treatment in the present study was not sufficient to cause maternal effects. Dispersal can be affected by competition for mates or resources in mammals [24,138,141,142], and dispersal is more likely where levels of competition are greater, as in high density environments [138]. Here, I provided cues of social competition to adult female bank voles to simulate relatively 'high' and 'low' levels of population density, but kept resource availability equal between treatment groups. Females could use the number of individuals present as a cue for the likely level of competition in the environment, and react accordingly to produce a maternal effect on offspring. However, maternal effects due to population density and social competition may instead result from an increase in the number of aggressive interactions or a decrease in resource availability, both of which should be expected where the level of competition is high. The results of the present study indicate that the number of individuals in the environment is insufficient to cause maternal effects on dispersal behaviour. Rather, other factors associated with high density, such as limited resource availability, may be necessary to cause maternal effects on offspring dispersal behaviour.

Lastly, differences in dispersal behaviour are likely to be most difficult to identify in the sex which is more likely to disperse, and this may have precluded the detection of a difference in male bank voles. In bank voles, males are generally more likely than females to disperse in

all populations tested [23,316,318–320,460]. Indeed, adult females may share territories with female offspring after weaning, but not with male offspring [23,311]. This indicates that male dispersal behaviour is relatively inflexible, as seen in other species of vole (e.g. the grey red-backed vole, *M. rufocanus* [483]). If this is the case, then maternal effects may not influence male dispersal behaviour, or the impact may be relatively subtle and thus difficult to detect. In total, 29 males were included in tests (high competition = 13, low competition = 16). It is possible that this sample size was insufficient to detect any subtle effect, although it is reasonably high for an animal behaviour study. If the sample size was insufficient, I would have expected an indication that the high competition males were generally bolder, but there no evidence for any such trend here. High competition males were on average quicker to enter the water, but slower to cross the water and enter the end chamber (Section 5.3.1.). Thus, whilst the data for entering the water indicates that high competition males are bolder, the data for entry to the end chamber suggests the opposite.

5.4.2. Maternal effects on dispersal behaviour in female bank voles

Previous studies have shown that mothers may produce offspring that are more likely to disperse or to disperse further when population density, and thus social competition, is high [403]. Thus, as above for males, I expected that the female offspring of females subject to cues indicating high levels of social competition would exhibit greater dispersal propensity and/or dispersal distance.

I inferred the relative dispersal behaviour of female bank voles born in different treatment groups by monitoring the movement of subjects in an outdoor semi-natural enclosure (25m x 40m). Dispersal behaviour of females could not be observed in the enclosure because the enclosure was too small to enable subjects to undergo movements of a similar length to those expected during dispersal (i.e. around 1km) [316,460]. I inferred dispersal behaviour from data on movements in the dispersal units, the relative number of captures near the outer edge of the enclosure, home range size, the maximum distance moved between consecutive captures and degree of overlap with maternal home range. In line with previous studies [473–475], I took more frequent entries to dispersal units, more captures near the outer edge of the enclosure, larger home ranges, longer movements or lower overlap with maternal home range to represent greater dispersal propensity and/or distance. However, as in studies with juvenile males, there was no evidence that dispersal behaviour of subjects differed between treatment groups. This suggests that social competition experienced by females did not influence the natal dispersal behaviour of their female offspring

The result is in conflict with expectations based on adaptive maternal effects on dispersal behaviour, and the results of similar previous studies. Where social competition is high, as in densely populated habitats, individuals may be excluded from resources that are required for survival and/or reproduction [250]. Maternal effects that drive dispersal from such areas are thus likely to be adaptive [250]. Indeed, high levels of social competition drive individuals to be more likely to disperse and/or to disperse further in several species [24,141,142,145,156,389]. Models by Fowler *et al.* [250] indicated that population density should cause adaptive maternal effects on dispersal behaviour. Moreover, population density and social competition in the maternal environment can influence offspring traits related to dispersal behaviour, including body size [411,438] and behavioural phenotypes [156,231].

Although the finding that there was no difference in dispersal behaviour in females born in different groups is inconsistent with most studies, it is consistent with some studies. Meylan *et al*. [403] found that population density in the maternal environment did not affect natal dispersal in *Lacerta vivipara*. The methods in that previous study differ markedly from those used here, so the results cannot be directly compared. In that study, the density of some populations, and thus the levels of social competition within them, were experimentally reduced by removing adult females. This means that neither control or 'reduced density' populations may represent a condition in which density, and thus level of social competition, is high. Nevertheless, the level of social competition would be higher in the control environment relative to the 'reduced density' environment, which may have been sufficient to cause a difference in dispersal behaviour. It is possible that in both that earlier study and the present one, the condition with the highest level of social competition was insufficient to cause increased dispersal propensity or dispersal in offspring produced in those environments.

The results related to the dispersal behaviour of female bank voles are also consistent with other findings in this project. In particular, I found no evidence that levels of corticosterone exhibited by adult females differed between groups (Section 4.3.1.). Previous studies have found that high levels of maternal corticosterone may cause increased dispersal propensity in offspring [396,398]. Thus, the lack of a difference in female dispersal propensity is consistent with the absence of any variation in faecal corticosterone concentration between groups. This comparison must be considered with caution, given that the reliability of corticosterone assays is uncertain (Section 4.3.1.).

Several factors may have prevented the detection of a maternal effect due to social competition. One possibility is that the treatment given to adult females was insufficient to cause a maternal effect. Several studies have found that population density can influence maternal effects on dispersal behaviour. Here, I specifically considered one aspect of population density; the number of individuals, and thus social competition. It is possible that this alone would not cause maternal effects on dispersal behaviour. The sample size used in the study may also have prevented the detection of a significant difference between treatment groups. In total, 29 females were weaned in the study. The number of females was heavily skewed towards those born in the high competition treatment group (22 high competition, 7 low competition). This skew may have prevented the detection of any difference in dispersal behaviour. However, if this were the case, I would expect a general indication from average values of measures of traits related to dispersal that dispersal behaviour differed overall between groups, and this is not the case here (see values in Section 5.3.2.).

Females may have exhibited a relatively low level of motivation to disperse because competition in the enclosures was relatively low and resource availability was high. Nelson *et al*. [467] used water-filled barriers to examine the emigration behaviour of wild male house mice. Individuals were found to traverse water when placed in an enclosure with poor quality habitat, but did not attempt to emigrate from a resource rich enclosure. The females in the present study were given an excess of food and water in the enclosure, and the majority of the enclosure was covered in vegetation for shelter. As such, the environment in the enclosure represents relatively good quality habitat, and motivation to leave may be low. If resources had been more limited in the population, a maternal effect may then have been evident.

It is possible that the study was insufficiently long to enable the detection of an effect. Many studies on dispersal take place across multiple seasons, with populations typically allowed to establish in a study site before experimentation (e.g. [465]). Here, females were only present in enclosures for seven weeks. Although shorter than most studies on dispersal behaviour, the present study is longer than other studies which examine the likelihood of animals to emigrate from an area. For example, the study by Nelson *et al.* [467] evaluated the propensity for emigration in *M. musculus* using seven-day long trials. The present study may therefore have been sufficiently long to enable the detection of an effect. However, a longer term study may have been beneficial, given that female bank voles may delay dispersal [23,318].

Female bank voles that disperse in the year of birth are likely to emigrate around the time of sexual maturity, and before mothers reproduce again [23,316]. Females may therefore have been expected to disperse within the study period. It is typical for females in the first litter of the year to disperse and for females in later litters to be relatively philopatric [23]. In the present study, animals were maintained indoors in conditions that were typical for spring and adult females had never previously mated. As such, juvenile females were expected to behave in a manner which was typical for the first litter of the year. However, female family groups were released in two different seasons; females from the first study block were released in early May, and those in the second block were released in mid-August. This could have caused females to display behaviour that is typical for that of offspring born in later litters of the year under natural conditions. The potential effect of study block was accounted for in models considering a maternal effect on female dispersal behaviour, so any difference due to block did not affect results. Future studies may benefit from examining the dispersal propensity of female bank voles born in enclosures during spring with differing levels of population density.

5.5. Conclusions

Maternal effects can act adaptively on dispersal behaviour by driving animals to be more likely to disperse or to disperse further when social competition is high. I investigated potential maternal effects on dispersal behaviour in males indirectly by evaluating boldness. Boldness is positively correlated with dispersal behaviour, with bolder individuals typically having greater dispersal propensity and/or dispersing further. I established that water could act as a barrier to movements in bank voles, and I used it to measure boldness. Contrary to expectations, I found no evidence that boldness varied between males born in different treatment groups. To investigate dispersal behaviour in females born in different treatment groups, I assessed their movements in an outdoor semi-natural enclosure over seven weeks. However, I found no evidence of a maternal effect on dispersal behaviour in female bank voles. Taken together, the results suggest that cues of social competition are insufficient to cause a maternal effect on dispersal behaviour in bank voles. However, further studies are required to ensure that a difference was not detected due to factors such as low sample sizes, the length of studies or the timing of studies.

Chapter 6: General discussion

Reproductive competition, dispersal behaviour, and the interactions between them, influence fundamental ecological and evolutionary processes. Understanding the causes and consequences of reproductive competition and dispersal, and how they interact, is therefore critical in applied fields such as species conservation, population control and maximising animal productivity [24,141–143,145].

Reproductive competition between males has received significant attention since it was first considered by Darwin [1,2]. Darwin [1,2] specifically focussed on pre-copulatory competition for mates, probably as this was the most overt expression of reproductive competition. Pre-copulatory competition has been the subject of many studies since the 1800s [9,43,47]. Post-copulatory competition became the focus of studies after 1970, when sperm competition was characterised by Parker [52]. Both pre- and post-copulatory competition in males have been studied extensively, and are now thought to be reasonably well understood. By contrast, reproductive competition between females has received relatively little attention [8]. The evolutionary consequences of reproductive competition between females remain poorly characterised, although several have now been identified [8].

Factors affecting dispersal and the consequences of dispersal are better studied in mammals than in any other taxa, except perhaps birds [24,141–143,145]. Although several potential proximate and ultimate causes of dispersal have been identified, its drivers remain poorly understood [142,145]. This is due largely to issues over definitions of dispersal, logistical difficulties involved in data collection and the historic absence of a phylogenetic framework [142,145].

Reproductive competition and dispersal behaviour are often discussed in isolation from one another, but they are highly interrelated. That is, dispersal may influence, and be influenced by, the nature and level of reproductive competition [24,141,142,196]. In this thesis, I have examined some key causes and consequences of dispersal and reproductive competition, and investigated potential links between them. I focussed particularly on the effects of the mating system, relatedness of individuals and maternal effects on reproductive competition and dispersal. I have employed phylogenetic methods and used a model species, the bank vole (*Myodes glareolus*), to test hypotheses for mammalian species, although my conclusions could be applied more broadly. In this final chapter, I summarise the results and implications of the research completed during this project. I will discuss the limitations of the work completed and potential future directions for research.

6.1. Mating system and dispersal behaviour

The level and nature of reproductive competition is closely associated with mating systems [12]. The mating system of a species is essentially the result of the strategies adopted by both males and females [12]. The strategies adopted by males are influenced by the defensibility of potential mates and the costs of locating them. Mate defensibility is influenced by the dispersion of sexually receptive females in space and time, which is related to resource availability [12,92]. In mammals, these factors account for a significant degree of the variation in mating behaviour both between and within species [12].

Although there is variation in the mating behaviour of species, and often between populations of a species [12,24], the mating systems of mammals are broadly classified as either polygyny, promiscuity, monogamy or polyandry. Each mating system is associated with different levels of reproductive competition, and thus different reproductive strategies. Several authors have hypothesised how, because of the associated differences in reproductive competition, the mating system of a species may be related to the dispersal pattern [24,141–143,145]. Associations between mating system and dispersal pattern have been proposed not only in mammals, but also for species in other taxa, most notably birds [24,141–143,145]. In mammals, it is expected that polygynous and/or promiscuous species will exhibit male-biased dispersal [24], and that monogamous species will have unbiased [141] or female-biased dispersal [24,145]. The hypothesised relationships between mating system and dispersal pattern have been widely accepted [142,145,196] and are central in dispersal theory. However, these hypotheses have only been tested using out of date methods without phylogenetic correction [24,141], confounded data [24,141] and/or relatively small datasets [24,141,145,193].

I constructed the largest database to date (n = 218) to determine whether the mating system of a species influenced the relative numbers of members of each sex which dispersed. I utilised the most recent mammalian supertree to control for the evolutionary relatedness of species, and used advanced comparative techniques to perform tests. I found little evidence to suggest that certain mating systems were associated with particular dispersal patterns in mammalian species (Chapter 2). Specifically, there was evidence of an association between male-biased dispersal and mating system, but only when polygyny and promiscuity were considered together. This is consistent with an early comparative study by Dobson [141], that considered the two mating systems together. However, there was no relationship when the mating systems were considered together, indicating that the earlier result may be

somewhat misleading. There was no association between female-biased dispersal and monogamy, although there was evidence for this in a phylogenetic study by Mabry *et al*. [145]. I found evidence that monogamy was related to equal dispersal, consistent with the predictions of Dobson [141], but the results suggest that monogamy did not precede equal dispersal as initially expected. These findings could be used as evidence that Greenwood [24] was correct to assert that the dispersal behaviour of a species or population was better explained by the 'ultimate causes' of dispersal (i.e. the levels of local mate, resource and/or kin competition and the need for inbreeding avoidance [142,196]) than it's mating system. Whilst the mating system of a species is closely associated with reproductive competition, the levels of competition for mates and other resources are not necessarily well approximated by mating system [24]. For example, although males in polygynous species may adopt mate-defence strategies, thus causing relative increases in local mate competition and driving male-biased dispersal, males do not adopt mate-defence strategies in all polygynous mammalian species [24]. If dispersal pattern is determined by the 'ultimate causes', then mating system may be a poor predictor of dispersal behaviour, as suggested by the results of this study.

These findings are crucial in dispersal theory, as they indicate that the dispersal propensity of members of each sex is not related to mating system in particular, consistent with the hypotheses initially outlined in Greenwood [24]. Future studies to progress this field could directly assess the effect of reproductive competition on the relative numbers of males and/or females which disperse. This would be challenging, given that the level of reproductive competition can be difficult to measure, but cross-species analyses would likely prove insightful. Future studies could also explore possible relationships between paternal care behaviour and dispersal. I found no evidence of a relationship between paternal provisioning behaviour and dispersal (Chapter 2). However, it is possible that paternal care and dispersal are related in one of many ways not considered here. For example, the relationship of monogamy and equal dispersal (ED) may be influenced by the presence of paternal care, with ED likely to evolve in monogamous species where males provide paternal care.

Although I focussed on mammals in this study, the results could be applied to species in other taxa. For example, dispersal patterns in birds may be explained with the same 'ultimate causes' proposed for mammalian species, which can be related to reproductive competition. Recent evidence from Mabry *et al*. [145] suggests that mating system and dispersal pattern

are not associated as expected in birds, consistent with the findings presented here for mammals.

This study could be extended if more data on mating system and dispersal propensity were available. However, given that many species were included in the analyses, the result is unlikely to be impacted by the addition of more species. The dataset used here may be biased towards the best studied species, but is expected to be representative of mammals in general. Data quality, and thus the reliability of results, could be improved if data on both mating system and dispersal were available on multiple populations of each species, although this would require significant research effort. The mammalian supertree used is the most accurate to date, but it contains many polytomies, so the estimated evolutionary relationships are unreliable. This study, as with any phylogenetic study on a large number of species, would be improved if the evolutionary relationships of species were better understood.

6.2. Relatedness of competing males and investment in reproductive strategies

Investment in different reproductive strategies is associated with characteristic phenotypic traits. For example, investment in pre-copulatory competition may cause increased body size (which can lead to sexual size dimorphism (SSD)), high levels of aggression and the development of armaments [63,153]. By contrast, investment in ejaculates is associated with, among other traits, enlarged testes relative to body size [80,81,102,117], more sperm in ejaculates [115,424] and the diversification of sperm morphology [85,108]. Investment in these different strategies, and thus the corresponding phenotypic traits, varies predictably according to mating system. For example, in polygynous mammalian species males compete primarily before copulation [24,153,226], whilst in promiscuous mammals most competition between males occurs post-copulation. The relatedness of competing males is also likely to affect the level of investment in particular reproductive strategies. Evidence from recent theoretical [117] and experimental studies [352,353,365] indicates that, for a given level of pre-/post-copulatory competition, males are likely to invest less when mate competition occurs between relatives.

In this study I explored the impact of the relatedness of males competing for mates on investment in both pre- and post-copulatory competition. Levels of investment in pre- and post- copulatory competition were inferred from measures of testes mass relative to body mass and sexual size dimorphism (SSD), respectively. I used data on male dispersal behaviour to deduce the relative risk that males would compete among kin, with the risk being greater

when brothers were likely to remain together. To account for differences in investment strategies due to mating system, I restricted analyses to promiscuous mammals.

6.2.1. Post-copulatory competition

Models by Parker [117] suggest that relatedness of males should influence investment in sperm competition. Males that compete for mates primarily among relatives are predicted to invest less in post-copulatory competition than those that compete mostly or entirely with non-kin. However, I found that ejaculate investment is actually higher when males are more likely to compete with kin for paternity (Chapter 3). The result does not necessarily conflict with Parker [117], as the higher level of investment could result because the risk of sperm competition is higher where kin are likely to compete. Indeed, males competing among kin are expected to be more likely to mate with a female that has previously mated [117]. It remains possible that, for a given risk of sperm competition, males invest less in their ejaculates when competing mostly among kin.

To my knowledge, this is the first comparative study to test the central hypothesis outlined in Parker [117] across mammalian species. Although I focussed on promiscuous species, the findings are key in gaining a greater understanding of the relationship between reproductive competition and dispersal behaviour in mammals, and possibly species in other taxa. The models by Parker [117] are not specific to mammals, so species in other taxa may exhibit similar trends to those observed in mammals. Indeed, the relatedness of male *Drosophila melanogaster* affects ejaculate investment in a manner consistent with the predictions from models by Parker [117] [352,353,365].

The results here are highly informative for future studies. For example, it is now evident that future studies would likely benefit from the incorporation of factors related to the risk of sperm competition, such as female mating frequency and male density. As data on such factors is currently limited, it would be necessary to take measurements on several species to complete such studies. Ideally, data on all factors incorporated in models would be collected from the same populations, but this will not be possible without the completion of a series of focussed studies on many species.

6.2.2. Pre-copulatory competition

Pre-copulatory contests are costly to males, not least because they can result in debilitating injuries and death [43]. The loss or incapacitation of kin during pre-copulatory contests may significantly, negatively affect the lifetime reproductive success of an individual. Individuals

can gain indirect fitness benefits where kin breed [218,219] and in some species, like lions (*Panthera leo*) [19], males may be dependent on kin to ensure access to mates. Males competing with close kin may thus be expected to minimise the risks associated with pre-copulatory competition, for example by instead investing more effort into post-copulatory competition.

I predicted that males competing among kin would invest less in pre-copulatory competition than those which usually compete with unrelated animals. I found that SSD (i.e. investment in pre-copulatory competition) and dispersal behaviour (i.e. relatedness of competing males) were associated. However, relatedness did not drive investment in pre-copulatory competition as expected. Instead, evolutionary transitions in dispersal behaviour occurred first. Specifically, the results indicate that males may alter dispersal behaviour to avoid competing with kin when the levels of pre-copulatory competition are high in promiscuous mammals. There was not strong support for this relationship, so it should be considered with care (Chapter 3).

Although the results do not constitute conclusive support for the initial hypothesis, they do provide interesting new insight. One reason for the low level of support for the hypothesis could be relatively small datasets (maximum number of species = 41) used in tests. If more species were included, then associations between pre-copulatory reproductive competition and dispersal may become more obvious. The level of data could be increased in future tests by including data from species with other mating systems, as long as the mating system was accounted for. Future studies may also benefit from the use of a different measure of investment in pre-copulatory competition. Male body size is related to investment in pre-copulatory competition, so I used SSD as a measure of investment in pre-copulatory competition [62,63,96,109]. However, the extent of SSD in a species is influenced by several factors, and SSD may not be an accurate measure of pre-copulatory competition. Indeed, reproductive competition may act to increase body size in both sexes [8], so a species may exhibit no SSD if both males and females are subject to high levels of reproductive competition. Alternative measures of male investment in pre-copulatory competition in future studies should reliably indicate investment and be comparable across species.

Whilst I focussed on mammals, there is no reason that the general theory applied here could not be applied to species in other taxa. The findings of this study could therefore be used as a basis for future studies across various species groups.

6.3. Reproductive competition as a cause of maternal effects in bank voles

I considered maternal effects to be any effect of the mother on offspring phenotype which is not solely genetically determined [248]. Although maternal effects can have negative impacts, they may also be adaptive, enabling matching between offspring phenotype and external conditions to maximise reproductive success of offspring [248]. In high density environments, competition for resources and mates is likely to be high [24,141]. The number of individuals in a population is likely to be a good indicator of the level of present and future competition. Differences in the number of animals encountered by mothers can induce maternal effects on several traits, including those associated with reproductive competition and dispersal (see e.g. [239,242,445]).

I used bank voles (*Myodes glareolus*) as a model to study how offspring phenotype may be influenced by the number of same-sex conspecifics encountered by the mother around the time of pregnancy. Adult females were divided equally between the 'high' and 'low' competition treatment groups. Females in both treatment groups were exposed to same-sex conspecifics using scent cues and direct encounters. High competition females were repeatedly exposed to three individuals, whilst low competition females repeatedly encountered only one other animal. I assessed the evidence for maternal effects on whole litters and on pups of each sex.

6.3.1. Maternal effects evident before weaning

I found no evidence that the number of individuals to which a mother was exposed caused maternal effects on litter size, pup growth rate until weaning or weaning weight of offspring (Chapter 4). I also detected no difference in corticosterone levels of adult females in different treatment groups before pregnancy, although (as discussed in Chapter 4) these results may not be reliable.

There was some evidence of a maternal effect on litter sex ratio. High competition females produced litters with more females relative to males compared to those produced by low competition females (Chapter 4). Females are the relatively philopatric sex in bank voles [23,318]. Thus, the result is contrary to expectations based on the local resource competition (LRC) hypothesis, which posits that females should produce lower numbers of the philopatric sex in high density populations [416]. Competition for resources, including mates, is relatively increased in high density habitats compared to those containing fewer conspecifics [138,389,437,484,485]. Production of the philopatric sex in densely populated habitats would further increase competition for resources, both between offspring of the philopatric

sex and between those offspring and their mother [416]. Previous studies have found that the reproductive fitness of female bank voles can be higher where they breed in kin groups [311]. This could have contributed to the relative over-production of females in simulated 'high density' conditions. However, a later study in this thesis showed that there was no effect of treatment group on the level of overlap between the home ranges of female offspring and their mothers (Section 5.3.2.). Future studies could explore whether there is evidence of a maternal effect on sex ratio in wild populations of bank voles with different levels of population density. If an effect is detected, studies should consider the adaptive significance of the skew in sex ratio in relation to the LRC hypothesis and the ecology of the bank vole.

Differences in litter sex ratio have been shown to result from differences in corticosterone [486], but there is no evidence for this here (Chapter 4). However, given that the results of the corticosterone assay may not have been reliable, further studies are required to determine whether corticosterone levels were likely to influence litter sex ratio in bank voles. Such studies should also consider the effects of other hormones such as testosterone and other molecules including glucose, both of which have been related to maternal effects in other vole species [254].

Although the reason for the effect on sex ratio is unclear, it is evidence that the methodology implemented was sufficient to cause maternal effects. As such, this finding can be considered a validation of the methodology used in this study, meaning that it is suitable for use in future studies on species which primarily use olfactory cues in communication.

6.3.2. Maternal effects on reproductive competition and dispersal behaviour

6.3.2.1. Male bank voles

In high density conditions, levels of reproductive competition (including local mate and resource competition) are likely to be greater than in low density populations. High levels of competition may lead to exclusion from resources and thus dispersal from an area [24]. Individuals are expected to invest more in reproductive competition in high density environments to ensure access to the resources required to breed [80,102,137,138,388,403,424,463]. If maternal effects are adaptive, then there should be evidence that they act to preadapt offspring to the environment in which they are born.

Bank voles are promiscuous, so males compete primarily post-copulation. If maternal effects in the present study were adaptive, I expected that males born in the high competition

treatment group should be better able to compete post-copulation. Accordingly, whilst there was no evidence of a maternal effect on traits associated with pre-copulatory competition (i.e. body size), ejaculate investment was greater in males born in the high competition treatment group (Chapter 4). Specifically, the combined mass of epididymides relative to body mass and daily sperm production were significantly greater for males born in high density conditions, but there was no evidence of a maternal effect on either testes mass or seminal vesicle mass. Taken together, these results provide evidence that, due to a maternal effect related to population density, males were preadapted to the likely level of reproductive competition. The differences detected here are not consistent with the expected effect of litter sex ratio on reproductive traits. Female-biased sex ratios are thought to result in the feminisation of males (see [274] and references therein), but this is not evident here.

I also examined boldness in male bank voles to indirectly assess whether the number of individuals in the maternal environment influenced their dispersal propensity (Chapter 5). I found no evidence of maternal effects on behaviours related to boldness (Chapter 5). Dispersal in bank voles is male-biased, and most males disperse from the natal area [23]. Habitual dispersal by males would have made any maternal effect on dispersal propensity difficult to detect, and this may have contributed to the absence of any difference in this study.

The evidence of a maternal effect on reproductive competition is consistent with the predictions of models of ejaculate investment under a varying risk of sperm competition. Ejaculate investment is expected to be increased where risk of sperm competition is greater, as anticipated with high population density, in order to maximise reproductive success (see e.g. [107]). However, previous studies of maternal effects on reproductive traits in bank voles suggest that high population density causes reproductive suppression [310]. The disparity between the result here and those in previous studies could be due to differences in methodology used to generate a maternal effect, and in the timing of measurements on reproductive traits. This highlights the importance of assessing traits using several methods, and monitoring effects through time in order to gain a fuller understanding on an effect. The results of the study assessing dispersal behaviour are also important, even though no difference was detected. Together, the findings could act as an example that, although reproductive competition and dispersal are thought to be closely related [24], direct links are not necessarily easy to detect. Longer-term, more detailed studies may reveal subtle

maternal effects in male dispersal propensity resulting from differences in population density.

The study could act as a basis for future studies of maternal effects on both reproductive traits and dispersal behaviour both in bank voles and in other species. One avenue of future study could be further assessment of ejaculate investment in relation to maternal effects. I assessed evidence for maternal effects on daily sperm production, but not the composition of ejaculates produced by males or investment in other components of the ejaculate. Studies could also explore potential causes of disparity between the results here and those suggesting that high density causes reproductive suppression. The cause of the disparity could be due to the difference in the treatment of adult females and/or the difference in the ages at which male reproductive traits were measured.

One of the main limitations involved in exploring maternal effects in this study was the sample size used in tests. The number of males was low because relatively few females produced litters and litter size was generally small. The sample size was further restricted by the high incidence of diabetes in the laboratory population of bank voles. Three males were excluded from statistical tests as there was evidence that investment in reproductive competition was negatively influenced by the presence of diabetes (Chapter 4). This problem may be lessened in future with the use of more females to produce litters. A greater understanding of how diabetes is transmitted and/or inherited in laboratory populations of bank voles should also help to increase sample sizes.

6.3.2.2. Female bank voles

Dispersal behaviour in female bank voles is more variable than that of males [23], so any maternal effects on dispersal should be more evident in females. I assessed evidence for maternal effects on the dispersal propensity of females by monitoring movements in semi-natural enclosures. I found no evidence of a maternal effect on dispersal behaviour, despite the use of several methods and intensive study (Chapter 5).

It is possible that a maternal effect of dispersal behaviour would have been detected in longer-term studies which included greater numbers of individuals, or where resource availability was lower. In other vole species with male-biased dispersal, specifically *Myodes rufocanus* (formerly *Clethrionomys rufocanus*), dispersal propensity in females was influenced by differences in sex ratio, with those from male-biased litters more likely to disperse [483]. There was no evidence to suggest that the differences in litter sex ratio

between groups had any effect in this study, but one may have been detected if dispersal behaviour could have been examined in more depth.

It is important to note that, whilst I cannot rule out that a difference would have been detected with the use of other methods, the results may simply indicate that there was no maternal effect on dispersal behaviour. The number of individuals regularly encountered by female subjects is one component of population density, and may be insufficient to cause a maternal effect on dispersal behaviour of offspring in isolation, even if it is sufficient to influence other traits (Section 4.3.). The number of individuals in a population can give some indication of likely levels of future competition. However, other factors related to high population density, such as increased resource competition, may be more important than the number of individuals encountered in driving maternal effects on dispersal. It is possible that maternal effects on dispersal would be evident if, for example, resource accessibility had differed between groups.

Although no difference was detected in this study, the findings provide insights which could be important in gaining a fuller understanding of maternal effects on dispersal. For example, the results indicate that studies determining how population density in the maternal environment can influence offspring phenotype should consider other factors besides the number of individuals present. These factors likely apply not only to bank voles, but also to other species of small mammal.

6.4. Concluding remarks

The research presented in this thesis constitutes a fundamental progression in our understanding of reproductive competition, dispersal behaviour and the links between them. This was achieved by testing hypotheses that have long been considered central in this area of study, including those initially outlined by Greenwood [24], Dobson [141] and Parker [117]. The findings of this research have also provided valuable insights on the impact of maternal effects on traits related to reproductive competition and/or dispersal. In particular, they show how population density in the maternal environment may influence the phenotype of offspring in a model species, and potentially other similar species. I focussed on mammalian species throughout the thesis, but the core principles from each study could be applied more broadly. This work further highlights that dispersal behaviour and reproductive competition are important and highly linked areas of study, and it provides a basis for many future studies to progress the field.

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Appendix 1: Information about the data used for tests in Chapter 2

The data used for tests in Chapter 2 is available on a USB stick which is in a pocket on the back cover of this thesis. Here I provide some information about the data file.

There are two sheets of the excel data file (Chapter 2 data.xls). The data is available on the first sheet, and references are provided on the second sheet. On the data sheet, the binomial names of species are provided in the column titled 'Species'. For some species, the genus name used in the literature from which data was taken differs from that used in the phylogenetic tree. Where this occurs, I have included both genus names in the data file. For example, in the literature the arctic fox is commonly referred to as '*Alopex lagopus*', but the species has recently been reclassified as '*Vulpes lagopus*', so I state the species name as '*Alopex/Vulpes lagopus*' in the data file. The dispersal pattern of a species is classified as either male-biased dispersal (MBD), female-biased dispersal (FBD), equal dispersal (ED) and high philopatry (HP). The mating system of a species is classified as promiscuity (Pr), polygyny (Po), monogamy (M) and/or polyandry (Pa). The 'confidence rating' refers to the confidence of the classifications of data. Confidence of classifications may be 'high' or 'low'. Data with 'high' confidence were included in the 'conservative' dataset (n=168 species) and those with 'low' confidence were included alongside 'high' confidence data in the 'all' dataset (n=218 species). See Chapter 2 for information on data classifications and details on the definitions of these terms. The numbers in the 'Reference for dispersal data' and 'Reference for mating system data' columns represent the reference(s) for data. All references are provided on the second datasheet (labelled 'References') in the data file.

Appendix 2: Transition rate combination values for models considering associations between mating system and dispersal behaviour

In Chapter 2, I investigated associations between dispersal patterns and mating systems in mammalian species. I found strong evidence of correlated evolution between male-biased dispersal (MBD) and a polygynous or promiscuous mating system when using the 'conservative' dataset to generate models. I also found evidence of correlated evolution between equal dispersal (ED) and monogamy when using either the 'all' or 'conservative' datasets to generate models. Here I present the transition rate combination values for the relationships detected in Chapter 2.

The transition rate combination shows which transitions are predicted to occur at the same rate, which transitions will occur at significantly different rates and which transitions are predicted not to occur. Transitions are predicted to occur at the same rate, or at least their rates are not predicted to be significantly different, if they are assigned the same numerical value (i.e. 0, 1, 2). Transitions which are predicted to occur at significantly different rates are assigned different numerical values. If a transition rate is predicted to be zero (i.e. the transition is not predicted to occur), then the transition is assigned a 'Z' value [1-3][146,335,337,340][146,335,337,340]. The values presented in Tables A2.1-3. were all generated in models which assumed that the evolution of two traits was correlated. However, it is possible for transition rate combinations which assume independent evolution to be 'visited' during a run [1-3]. Transition rate combinations which are 'visited' more frequently in the post-convergence portion of the run are more likely to represent the true rate combination (i.e. how traits coevolved).

Table A2.1. Transition rate combinations generated using the conservative dataset for the evolution of male-biased dispersal (MBD) and polygyny (Po) or promiscuity (Pr) in mammals. Rank represents the relative likelihood of a combination, with higher ranked combination observed more frequently. The ten most frequently observed transition rate models are shown, the fiftieth most frequently observed value is included for comparison. The model string indicates the transition rate combination. The transition rates are presented in the following order: q12, q13, q21, q24, q31, q34, q42, q43. The transition rate combination may represent either a dependent (D) or an independent (I) model of evolution. Occurrence frequency shows the frequency with which a combination was observed in the post-convergence portion of the run.

Rank	Model string	Model of evolution	Occurrence frequency
1	'0 0 0 0 0 0 0 Z	D	111
2	'0 1 1 1 0 1 1 Z	D	78
3	'1 0 0 0 1 1 0 Z	D	62
4	'1 0 0 0 1 0 0 Z	D	62
5	'0 1 1 1 0 Z 1 Z	D	52
6	'0 1 1 1 0 0 1 Z	D	51
7	'1 0 0 0 1 Z 0 Z	D	41
8	'1 1 0 0 1 1 0 Z	D	31
9	'0 0 1 1 0 0 1 Z	D	27
10	'1 1 1 1 1 0 1 Z	D	24
50	'0 1 1 0 0 Z 0 Z	D	4

Table A2.2. Transition rate combinations generated using the all dataset for the evolution of equal dispersal (ED) and monogamy (M) in mammals Rank represents the relative likelihood of a combination, with higher ranked combination observed more frequently. The ten most frequently observed transition rate models are shown, the fiftieth most frequently observed value is included for comparison. The model string indicates the transition rate combination. The transition rates are presented in the following order: q12, q13, q21, q24, q31, q34, q42, q43. The transition rate combination may represent either a dependent (D) or an independent (I) model of evolution. Occurrence frequency shows the frequency with which a combination was observed in the post-convergence portion of the run.

Rank	Model string	Model of evolution	Occurrence frequency
1	'Z 0 Z 1 1 0 1 1	D	127
2	'Z 0 0 1 1 0 1 1	D	86
3	'Z 1 Z 0 0 1 0 0	D	80
4	'Z 0 Z 1 1 0 0 1	D	59
5	'Z 0 1 1 1 0 1 0	D	51
6	'Z 0 0 1 1 0 1 0	D	51
7	'Z 1 1 0 0 1 0 1	D	36
8	'Z 1 Z 0 0 1 1 0	D	31
9	'Z 0 Z 0 1 0 0 1	D	28
10	'Z 0 Z 1 1 0 1 0	D	23
50	'Z 1 0 1 0 1 0 0	D	3

Table A2.3. Transition rate combinations generated using the conservative dataset for the evolution of equal dispersal (ED) and monogamy (M) in mammals. Rank represents the relative likelihood of a combination, with higher ranked combination observed more frequently. The ten most frequently observed transition rate models are shown, the fiftieth most frequently observed value is included for comparison. The model string indicates the transition rate combination. The transition rates are presented in the following order: q12, q13, q21, q24, q31, q34, q42, q43. The transition rate combination may represent either a dependent (D) or an independent (I) model of evolution. Occurrence frequency shows the frequency with which a combination was observed in the post-convergence portion of the run.

Rank	Model string	Model of evolution	Occurrence frequency
1	'Z 0 Z 1 1 0 1 1	D	317
2	'Z 1 Z 0 0 1 0 0	D	92
3	'Z 0 0 1 1 0 1 0	D	91
4	'Z 1 1 0 0 1 0 1	D	63
5	'Z 0 0 1 1 0 1 1	D	50
6	'Z 0 Z 1 1 0 1 0	D	34
7	'Z 0 1 1 1 0 1 0	D	29
8	'Z 1 Z 0 0 1 0 1	D	24
9	'Z 1 1 0 0 1 0 0	D	18
10	'Z 1 1 1 0 1 0 1	D	18
50	'Z 0 1 1 1 2 1 1	D	1

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Appendix 3: Data for comparative analyses in Chapter 3

Table A3.1. Most reliable data included in the 'conservative' dataset and in the 'all' dataset alongside lower confidence data.

Species	Male body mass (g)	Combined testes mass (g)	Relative risk of intra-kin competition	References
Acinonyx jubatus	43700	15.51	High	1, 2, 3
Aepyprymnus rufescens	2400	4.68	Low	4, 5
Antechinus minimus	47.25	1.068	Low	6, 7, 8
Antechinus stuartii	33.993	0.41	Low	4, 6, 9, 10, 11, 12
Antechinus swainsonii	124.85	0.281	Low	4, 11, 12
Brachyteles arachnoides	9600	990.48	High	13, 14, 15
Cebus capucinus	3500	5.146	High	16, 17, 18
Cebus nigritus	3466.667	7.613	Low	1, 2, 9, 19, 14, 20, 21
Delphinapterus leucas	755061.939	571.04	Low	22, 23
Dipodomys merriami	38.25	0.45	Low	10, 19, 20, 24
Grampus griseus	470000	14100	High	22, 25, 26
Lagothrix lagotricha	5220	11.2	High	10, 14, 19, 27
Lemur catta	2700	17.8	Low	2, 28
Lepus americanus	1300	11.8	Low	29, 30
Lepus timidus	2635	16.66	Low	10, 31
Marmota monax	4081.333	7.4	Low	1, 10, 19, 32
Microcebus murinus	67.22	2.27	Low	1, 2, 14, 19, 29, 33
Mungos mungo	150	1.76	High	34, 35
Myodes rufocanus	39	0.161	Low	29, 36
Nasua narica	4400	4.84	Low	1, 37
Neovison vison	1603	2.490	Low	7, 29, 38
Niviventer coning	192	4.77	Low	39, 40
Otolemur crassicaudatus	1563.5	7.552	Low	2, 14, 41
Ovis Canadensis	95750	218	Low	1, 2, 7, 42
Pan paniscus	42050	192.6	High	1, 2, 29, 43, 44
Pan troglodytes	44320	118.4	High	1, 2, 9, 10, 14, 19, 20, 29, 43, 45, 46
Panthera leo	165325	45.867	High	1, 19, 47, 48
Panthera tigris	262292.5	24.4	Low	1, 19, 49
Peromyscus maniculatus	20.225	0.393	Low	2, 9, 10, 19, 20, 29, 50, 51
Pongo abelii	105700	34.87	Low	43, 52
Rattus lutreolus	139.617	4.43	Low	2, 19, 20, 53
Semnopithecus entellus	17075	11.15	Low	7, 54
Spermophilus beldingi	316.5	3	Low	19, 55, 56, 57
Tylonycteris pachypus	4.1	0.14	Low	58, 59
Ursus americanus	113643.5	47.45	Low	1, 29, 60
Ursus arctos	226590	106.867	Low	1, 2, 19, 20, 61
Xerus inauris	715.09	10.01	Low	62, 63

Table A3.2. Least reliable data included in the 'all' dataset

Species	Male body mass (g)	Combined testes mass (g)	Relative risk of intra-kin competition	References
Alouatta caraya	6420	18.37	High	1, 64
Ateles fusciceps	2600	9.1	High	1, 65
Ateles geoffroyi	7940	13.4	High	1, 19, 65
Macaca radiata	7125	7.22	Low	7, 66
Rattus fuscipes	122.85	4.262	Low	2, 19, 67
Tamiasciurus hudsonicus	244	0.158	Low	2, 68, 69
Tursiops aduncus	200000	2000	Low	1, 70

References for sperm competition data: (1) Lüpold (2013) (2) Tourmente *et al*. (2011) (3) Caro and Collins (1987) (4) Taggart *et al.* (1998) (5) Pope *et al*. (2005) (6) Taggart *et al*. (2003) (7) Anderson and Dixon (2009) (8) Magnusdottir *et al*. (2008) (9) Gage and Freckleton (2003) (10) Kenagy and Trombulak (1986) (11) Rose *et al*. (1997) (12) Cockburn *et al*. (1985) (13) Lemos De Sá and Glander (1993) (14) Wong (2014) (15) Printes and Strier (1999) (16) Crofoot *et al*. (2009) (17) Jack and Fedigan (2004) (18) Wikberg *et al*. (2014) (19) Lemaître *et al*. (2009) (20) Ramm (2007) (21) Janson *et al*. (2012) (22) Kelley *et al*. (2014) (23) O'Corry *et al*. (1997) (24) Zeng and Brown (1987) (25) Chen *et al*. (2011) (26) Hartman *et al*. (2008) (27) Nishimura (2003) (28) Sussman (1992) (29) Soulsbury (2010) (30) Burton *et al*. (2002) (31) Bray *et al*. (2007) (32) Maher (2009) (33) Radespiel *et al*. (2003) (34) Anderson *et al*. (2004) (35) Nichols *et al.* (2012) (36) Ims (1989) (37) Gompper *et al.* (1998) (38) Zalewski *et al*. (2009) (39) Yu and Lin (1999) (40) Wu and Yu (2000) (41) Clark (1985) (42) Buchalski *et al*. (2015) (43) Dixson (2012) (44) Eriksson *et al*. (2006) (45) Lagergraber *et al*. (2009) (46) Mitani *et al*. (2002) (47) Packer and Pusey (1982) (48) Pusey and Packer (1987) (49) Smith (1993) (50) Yang and Kenagy (2009) (51) King (1986) (52) Nater *et al*. (2013) (53) Stephens *et al*. (2013) (54) Borries (2000) (55) Nunes *et al.* (1997) (56) Holekamp *et al*. (1984) (57) Holekamp (1986) (58) Hua *et al.* (2013) (59) Wilkinson and McCracken (2003) (60) Costelle *et al*. (2008) (61) Jerina, K. and Adanič, M. (2008) (62) Waterman (1995) (63) Scantlebury *et al*. (2008) (64) Oklander *et al*. (2010) (65) Lawson Handley and Perrin (2007) (66) Cooper *et al*. (2004) (67) Peakall *et al*. (2003) (68) Berteaux and Boutin (2000) (69) Haughland and Larsen (2004) (70) Möller and Beheregaray (2004).

Table A3.3. Body weights of sexually mature adults used to calculate sexual size dimorphism (SSD). All body weights taken after sexual maturity.

Species	Male mass (kg)	Female mass (kg)	Refs
Acinonyx jubatus	52.75	44.25	1
Aepyprymnus rufescens	3	3.5	1
Alouatta caraya	6.61	4.468	1
Antechinus minimus	0.065	0.042	1
Antechinus stuartii	0.039	0.026	1
Antechinus swainsonii	0.063	0.0432	1
Ateles fusciceps	8.89	8.98	2, 3
Ateles geoffroyi	7.995	7.373	2. 3
Brachyteles arachnoides	10.868	8.76	1, 2, 3
Cebus capucinus	3.774	2.603	2, 3
Cebus nigritus	3.35	2.458	2, 3
Delphinapterus leucas	947	661	1
Dipodomys merriami	0.032	0.036	1
Lagothrix lagotricha	7.632	6.600	1, 2, 3
Lemur catta	2.555	2.355	2, 3
Lepus americanus	1.38	1.42	1
Lepus timidus	2.7	3	1
Macaca radiata	6.59	3.747	1, 2, 3
Marmota monax	3.1	3.08	4
Microcebus murinus	0.073	0.074	2, 3
Mungos mungo	1.131	1.242	1
Myodes rufocanus	0.036	0.043	4
Nasua narica	5.1	3.7	5
Neovison vison	1.15	0.6	3
Niviventer coninga	0.18	0.14	6
Otolemur crassicaudatus	1.227	0.993	1, 2
Ovis canadensis	137.292	94.702	1, 3, 7
Pan paniscus	42	32.1	2, 3
Pan troglodytes	49.3	40.533	1, 3
Panthera leo	187.333	139.5	1
Panthera tigris	216	134	1
Peromyscus maniculatus	0.020	0.020	1
Pongo abelii	77.9	35.6	2
Rattus fuscipes	0.115	0.1	1
Rattus lutreolus	0.13	0.12	1
Semnopithecus entellus	13	9.89	2
Spermophilus beldingi	0.22	0.218	4
Tamiasciurus hudsonicus	0.235	0.225	1, 4
Ursus americanus	172.333	90.067	1
Ursus arctos	227	182	1
Xerus inauris	0.536	0.582	1

References for dimorphism data; (1) Silva and Downing (1995) (2) Smith and Jungers (1997) (3) Weckerly (1998) (4) Schulte-Hostedde (2007) (5) Gompper (1996) (6) Yu and Lin (1999) (7) Mysterud (2000).

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